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SOMATIC CROSSING OVER AND SEGREGATION IN DROSOPHILA MELANOGASTER*

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Received May 6, 1936

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INTRODUCTION

IN 1925 BRIDGES found that females of *Drosophila melanogaster* containing the dominant factor Minute-n in one X chromosome and some recessive genes in the other X chromosome often exhibit a mosaic condition. While the main surface area of these flies showed the effect of the dominant Minute-n (I, 62.7) without the effect of the recessive genes, as was to be expected, smaller areas, in different regions of the body and of varying size, were not Minute-n, phenotypically, but displayed the effects of the recessive genes. BRIDGES' interpretation was this: the Minute-n factor has the property to eliminate occasionally the X chromosome in which it itself is located. The cells of mosaic spots are descended from one common ancestral cell in which such elimination had taken place. They possess, therefore, only one X chromosome and show the phenotype produced by its genes.

Minute-n is only one of a group of factors which are very similar in their phenotypical expression. The "Minutes" behave as dominants whose most striking phenotypic effect is a reduction in bristle size; in addition there is a strong retardation in development, tendency to rough eyes, etc. The homozygous Minute condition is lethal. Some Minutes have been shown to be deficiencies (SCHULTZ 1929). Many Minute factors have been found in different loci of all chromosomes. They are distinguished by adding different letters or numbers to the symbol *M*.

Following BRIDGES' discovery of mosaics with respect to sex-linked factors, the appearance of mosaic spots which exhibit autosomal characters was described (STERN 1927b). Such spots appear on flies which originally had a constitution heterozygous for genes determining the characters. These mosaics occurred in crosses in which autosomal Minute factors were present and the facts seemed to agree with the interpretation that the spots were due to an elimination of that arm or part of an autosomal chromosome which carried the Minute.

The present investigation was originally designed to attack the problem: How is a Minute factor able to eliminate the chromosome or that part of a chromosome in which it itself is located?

At the same time the solution of another problem was sought. The fact that small mosaic spots showed the phenotypic effect of certain genes contained in their cells whereas the remainder of the individual showed another phenotype was proof of the autonomous development of these char-

acteristics. Among the very few genes which did not show phenotypical effects in spots was the recessive "bobbed" (I, 66.0) which produces short bristles: in $+^{y}Mn+^{bb}/y+^{Mn}bb$ females the $y+^{M}$ spots did not possess the *bb*-type bristle length, but a $+^{bb}$ length. Non-autonomous development of the bobbed character seemed improbable as typical gynandromorphs had shown clear demarcation lines for the *bb* and $+^{bb}$ areas (STERN 1927a). As bobbed is located at the extreme right end of the genetic X chromosome, next to the spindle fibre attachment, the following hypothesis was proposed: just as in the case of autosomal eliminations only part of the autosome disappears, so also in *Mn* mosaics merely a portion of the X chromosome is eliminated. The piece adjoining the spindle attachment and including the bobbed locus is assumed to be left in the cell, thus giving a constitution $+^{bb}/y+^{Mn}bb$, which, being heterozygous for *bb*, does not produce the effect of this gene (STERN 1928b). When PATTERSON (1930) using MULLER'S Theta translocation showed that in his cases of X-radiated flies "not the whole X chromosome was eliminated" it was decided to use the same genetic technique to test the above hypothesis as to the partial elimination in the case of *Mn*. The Theta translocation was kindly put at my disposal by Prof. H. J. MULLER.

Both problems, the question as to the action of Minutes to bring about elimination and the question as to complete or partial elimination of the X chromosome (intimately bound up with the question as to autonomous or non-autonomous development of bobbed in small spots), proved to be based on an erroneous concept as to the origin of mosaic spots. While the investigation revealed this, it provided at least a partial solution of the problems by the discovery of somatic crossing over and segregation in Drosophila.

ACKNOWLEDGMENTS

Some of the work with autosomal Minutes was carried out in 1926 at the Department of Zoology of Columbia University in New York City under a Fellowship from the International Education Board. These experiments were continued at the Kaiser Wilhelm Institut für Biologie in Berlin-Dahlem. The greater part of the investigation proceeded since December 1932 in the following institutions: Kerckhoff Biological Laboratories, California Institute of Technology, Pasadena, California (Fellow of the Rockefeller Foundation); Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. (guest); and Department of Zoology, University of Rochester, Rochester, N. Y. The author is grateful for all the help given him everywhere, especially for the very generous hospitality at Cold Spring Harbor extended to him in a difficult time by Prof. C. B. DAVENPORT and Dr. M. DEMEREC.

For suggestions concerning the manuscript thanks are due to members of the Biological Laboratories in Pasadena and to my wife, EVELYN STERN.

METHODS

The methods were similar to those used by BRIDGES. Flies were made heterozygous for recessive genes whose phenotypic effects were of such a kind as to be exhibited by very small areas, preferably even single setae. The setae of Drosophila are divided into macrochaetae and microchaetae, the former generally called bristles, the latter, hairs. As far as the purpose of the present study is concerned the distinction is of no intrinsic importance. Genes mainly used were: (a) yellow body-color (*y*, 1, 0.0), producing an effect which can be distinguished in a single hair, making it yellowish-brownish as opposed to the black not-yellow condition (the general coloring effect of *y* on the hypodermis is often not very distinct in spots (STURTEVANT 1932)) and (b) singed-3 (sn^3, 1, 21.0), producing a thickened, curved or crooked condition of the setae, which generally can be distinguished in single hairs also. However, doubts occasionally remain as to whether a single hair on a heterozygous $+/sn^3$ fly is genotypically singed or whether it is normal but slightly more bent than usual. With spots of two or more hairs such doubts hardly ever occur.

Following BRIDGES, the flies were inspected originally for spots only on the head and thorax. In later experiments, however, inspection of the abdomen was included. In order to discover even the smallest spots the flies were scrutinized under a binocular magnification of 37× (Bausch & Lomb objective 3.7, eyepieces 10×). The use of a simple device made by the Bausch & Lomb Optical Company which allows for the fine adjustment to be made by foot movements and leaves both hands free for manipulation, proved to be of great value (for more detailed description see Drosophila Information Service 6:60).

As the study of spots was practically confined to setae-bearing regions, a table of the number of setae on different parts of the body of average sized females was computed. Generally only the dorsal and lateral parts of the thorax and only the tergites of the abdomen were inspected and, except in special cases, no effort was made to remove the wings in order to uncover completely the median part of the abdominal tergites. This partly covered region excluded about 20 per cent of the abdominal setae from inspection (table 1).

A separate record was kept for each spot consisting of an outline drawing in case of head and thorax mosaics or of a notation of number of macro- and microchaetae and position in case of abdominal mosaics.

Spots on the dorsal side of the thorax nearly always form one single clearly defined mosaic area, while spots on abdominal tergites are fre-

TABLE 1

Number of setae on different body regions.

	HEAD*	THORAX*	ABDOMINAL TERGITE					
			1 AND 2	3	4	5	6	7
Mean number of setae	34	238	108	120	117	123	120	61
Not inspected (estimate) %	0	0	50	40	20	0	0	0
Inspected	34	238	54	72	94	123	120	61
% of total inspected setae	4	30			66			
Inspected abdominal setae (% of total)			10	13	17	24	24	12

Total setae inspected: ca. 800.
Total abdominal setae inspected: ca. 525.
* Dorsal parts only.

quently broken up into two or more separate parts. Apparently the growth processes in the imaginal discs of thorax and abdomen are somewhat different.

THE ACTION OF MINUTE FACTORS

Blond-Minute

As stated in the introduction, a number of Minute determiners have been found to be deficiencies for short regions. The hypothesis suggested itself that the apparent tendency of Minutes to eliminate the chromosomes or chromosomal parts in which they are located is due to some mechanical disturbance of chromosome division which itself is caused by the material defect in the deficiency chromosome.

In order to test this hypothesis use was made of the "Blond-translocation" (Bld) which represents a reciprocal translocation between the left end of the X chromosome and the right end of the second chromosome (BURKART and STERN 1932). When all chromosomes of an individual are balanced with respect to the translocation, normal sized Blond bristles are produced. However, in females if one X chromosome lacks its extreme left end without being compensated for by the presence of the translocated piece on the second chromosome, then the individuals possess bristles of Minute, Blond character. BURKART had found that these Minute females

show mosaic spots, a fact which seemed to indicate that elimination of the deficient X chromosome occurred.

The viability of Blond-Minutes is rather low and depends greatly on culture conditions. In half-pint milk bottles with one pair of parents their viability was found to average 30 per cent of their not-Minute sisters. Under PATTERSON'S culture conditions the Minutes apparently never appeared and this led him to assume a viability factor in the region covered by the deficiency (PATTERSON 1932). The absence of this factor was believed to cause the death of the zygote.

In a Blond male the left end of the X is translocated to the right end of one of the second chromosomes. Such a male forms two kinds of X carrying gametes: (1) deficiency X, translocation II; (2) deficiency X, normal II. Mated to a normal female, two kinds of daughters are produced: (1) def./normal; transl. II/normal II; (2) def./normal; normal II/normal II. The females (1) are not-Minute, the females (2) are Minute. *If elimination of the deficient X chromosome is due to the deficiency itself then the percentage of eliminations should be equal in both classes.* Accordingly, Blond males were mated to females with morphologically normal chromosome constitution homozygous for singed-3. Elimination of the deficient chromosome was deduced from the appearance of not-Blond, singed setae mainly on head or thorax. Out of 323 Blond-Minute females (2) 143 had one or more such mosaic spots making a total of 167 (to which should be added 12 spots of somewhat different constitution cf. table 9). In 923 Blond not-Minute sisters (1) not a single spot was exhibited. This result proves that the deficient consitution of the Blond X chromosome itself is not the cause of eliminations, and suggests that eliminations of the X chromosome are due to the phenotypic "Minute reaction" (SCHULTZ 1929), that is, to the same or part of the same physiological condition which results in the development of a short-bristled late hatching "Minute" fly.

One difficulty, however, remained. Why should the "physiological Minute condition" eliminate only the deficient X chromosome? That only the deficient X was affected and not its normal partner seemed to be indicated by an experiment in which the sn^3 gene was located together with Bld in the deficient X, the other X containing white (*w*, I, 1, 5) or other recessives. In 221 def. Bld sn^3/w; normal II/normal II Minute females, 94 bristles containing spots on head and thorax were found, all not-Bld, not-sn^3. They seemed to result from eliminations of the deficient chromosome, while the absence of sn^3 spots seemed to exclude the elimination of the not-deficient X chromosome. Was there such an interaction that the physiological Minute condition produced by an uncovered deficiency in the X would eliminate just this deficient chromosome which was quite

stable when its phenotypic effect was suppressed by the normal allele for that region translocated to the second chromosome?

Autosomal Minutes and sex-linked spots

When the ability of autosomal Minutes to produce "autosomal" mosaics had been discovered, the following cross was made in order to detect a possible influence of such Minutes on the appearance of sex-linked mosaics: sn^3/sn^3 ♀ by $+^{sn}$; $My/+$ ♂ (*My*, Minute-y, III, 40.4). While 1020 $+^M F_1$ females had 3 single-bristle singed spots, 811 *My* F_1 sisters exhibited 16 spots (13 single-bristle, 3 larger ones, table 2a, first row). One of the spots in a $+^M$ fly occurred on the abdomen and is not included in the table. This result showed (1) that mosaic spots appear as rare occurrences even in not-Minute flies (BRIDGES in MORGAN, STURTEVANT, BRIDGES 1929, has encountered four such cases in his experiments) and (2) that an autosomal Minute increases the frequency of these occurrences, that is, is able to influence the fate of an X chromosome.

Later work confirmed this finding and has led to the use of autosomal Minutes as tools in the study of sex-linked mosaics.

Besides *My* three other autosomal Minutes have been tested for their effect on the X chromosome behavior, namely *Mw* (Minute-w, III, 80±), *M33j* (Minute-33j, III, 40.4) and *Mβ* (Minute-β, III, 85.4). Table 2a shows the results of some tests. It should be pointed out that the constitution of the X chromosomes varied in these experiments, so that the frequencies of spots in different experiments are not comparable. In some experiments only the head and thorax were inspected for spots. In all later tests the abdomen was inspected also. Due to the comparatively small size of the head and the low number of setae the number of head spots has been added to the number of thorax spots under one grouping. An inspection of tables 2a, b shows, among other results:

(1) The frequency of sex-linked spots in not-Minute flies varied from 0.0 to 6.0 on the head-thorax region and from 4.6 to 20.0 per cent on the abdomen. The frequency in Minute flies varied from 0.0 to 22.3 in the head-thorax region and from 8.0 to 36.6 per cent on the abdomen.

(2) A positive correlation is indicated between percentage frequency of head-thorax and abdominal spots:

(a) In not-Minute flies

experiment	*Mw*(5)	*Mβ*	*M33j*(3)	*M33j*(2)	*Mw*(4)	*M33j*(1)
frequency on head-thorax	0.0	0.7	0.8	1.4	4.5	6.0
frequency on abdomen	15.0	4.6	8.2	9.2	11.7	20.0

(b) In Minute flies

experiment	*Mw*(5)	*Mβ*	*Mw*(4)	*M33j*(3)	*M33j*(1)	*M33j*(2)
frequency on head-thorax	0.0	3.1	4.4	5.7	16.8	22.3
frequency on abdomen	27.1	8.0	28.1	33.4	36.6	15.9

Two of the discrepancies in these series seem to be based on a special condition which hindered the appearance of all head-thorax spots. This experiment (*Mw*(5)) therefore is not comparable with the rest.

(3) The relative increase of spots in Minute as compared to not-Minute flies varies from 1.0 to 15.6 times in the head-thorax and from 1.7 to 4.1 times in the abdominal region.

(4) The average increase is distinctly lower in the abdominal region.

(5) As far as the data are significant, no correlation seems to exist between amount of increase of spots in the two different body regions:

experiment	*Mw*(4)	*M33j*(1)	*Mβ*	*M33j*(3)	*M33j*(2)
increase on head-thorax	1.0	2.8	4.7	7.2	15.6
increase on abdomen	2.4	1.8	1.7	4.1	1.7

The data of tables 2a,b had shown the effect of autosomal Minutes on the *frequencies* of occurrence of sex-linked spots. Is there also an influence

TABLE 2a

Frequency of sex-linked spots in Minute and not-Minute females.

MINUTE USED AND NO. OF EXP.	INDIVIDUALS INSPECTED		HEAD-THORAX SPOTS					ABDOMINAL SPOTS				
			NO.		%		%	NO.		%		%
	+	*M*	+	*M*	+	*M*	*M*:+	+	*M*	+	*M*	*M*:+
My†	1020	811	2	16	.2	2.0	10.5	—	—	—	—	—
Mw (1)†	964	813	8	23	.8	2.8	3.4	—	—	—	—	—
(2)†	377	284	7	17	1.9	6.0	3.2	—	—	—	—	—
(3)†	151	119	3	20	2.0	16.8	8.5	—	—	—	—	—
(4)	154	114	7	5	4.5	4.4	1.0	18	32	11.7	28.1	2.4
(5)‡	432	247	0	0	0	0	—	65	67	15.0	27.1	1.8
M33j (1)	135	131	8	22	6.0	16.8	2.8	27	48	20.0	36.6	1.8
(2)	349	251	5	56	1.4	22.3	15.6	32	40	9.2	15.9	1.7
(3)*	377	296	3	17	.8	5.7	7.2	31	99	8.2	33.4	4.1
Mβ	307	227	2	7	.7	3.1	4.7	14	18	4.6	8.0	1.7

† Abdomen not inspected.
‡ No head-thorax spots present.
* No head spots present.

on *time* of occurrence in development of the process which leads to appearance of a mosaic spot? The earlier this time is, the larger the spot should be. Accordingly, in table 3 the spots are divided into three groups, those

TABLE 2b

Proportion of number of head-thorax:abdominal spots in $+^M$ and M females.

EXP.	NUMBER OF HEAD-THORAX AND ABDOMINAL SPOTS +	M
Mw	7– 18	5– 32
M33j (1)	8– 27	22– 48
(2)	5– 32	56– 40
(3)	3– 31	17– 99
Mβ	2– 14	7– 18
Total	**25–116**	**107–237**

χ^2 about 10. P<.01.

comprising only one seta, only two setae, and more than two setae. This last class includes all spots from those involving three setae up to those occasional spots which include all setae of the imaginal disc concerned. The data are comparatively meager, but the following generalizations seem to be warranted:

(1) Most experiments agree in the relative frequencies of small and larger spots in that the one seta spots form the majority both on the head-thorax and on the abdominal region. (2) Some striking exceptions to this occur, notably the head-thorax spots in Minute flies of *Mw*(2) and (3) and the abdominal spots in both not-Minute and Minute flies of *M33j*(1). Here the frequencies of spots larger than one seta is higher than that of one seta spots: 14:3; 16:4; 20:7; 36:22. (3) The proportion of

TABLE 3

Frequency of sex-linked spots of different sizes in the experiments described in Table 2a.

EXP	HEAD-THORAX							ABDOMEN							
	NO. OF SPOTS		SIZE OF SPOTS						NO. OF SPOTS		SIZE OF SPOTS					
	+	M	+			M			+	M	+			M		
			1	2	>2	1	2	>2			1	2	>2	1	2	>2
My	2	16	2	—	—	13	1	2	—	—	—	—	—	—	—	—
Mw (1)	8	23	5	—	3	16	2	5	—	—	—	—	—	—	—	—
(2)	7	17	3	2	2	3	3	11	—	—	—	—	—	—	—	—
(3)	3	20	3	—	—	4	3	13	—	—	—	—	—	—	—	—
(4)	7	5	5	—	2	4	—	1	18	32	17	—	1	13	10	9
(5)	0	0	0	0	0	0	0	0	65	67	41	17	7	42	11	14
M33j (1)	8	22	6	1	1	13	4	5	27	48	7	6	14	22	11	25
(2)	5	56	5	—	—	42	4	10	32	40	17	4	11	25	6	9
(3)	3	17	3	—	—	16	—	1	31	99	19	6	6	60	17	22
Mβ	2	7	2	—	—	2	—	5	14	18	9	2	3	9	2	7

single seta spots is lower in the abdomen than in the head-thorax region. (4) In most experiments no striking influence of the Minute on the time of occurrence of the spot-producing process can be found.

The ontogenetic meaning of some of these findings will be dealt with later.

The specificity of the effects of sex-linked and autosomal Minutes

In view of the effect of autosomal Minutes on the X chromosome it seemed desirable to test for a possible influence of an X chromosome Minute on the behavior of autosomes. The Blond Minute is known to be one of the most potent factors for the production of sex-linked spots and accordingly was chosen for the test in regard to autosomal spots. Females of the constitution *h st cu sr* e^s *ca* (located over most of the length of chromosome III) were mated to Blond males and their Minute daughters were inspected for a condition mosaic for *h*, *st*, *sr*, e^s or *ca*. Although the 413 F_1 females exhibited on head and thorax 94 mosaic conditions for Blond, none was found to possess an autosomal spot. This shows that the influence of Blond-Minute on the production of autosomal mosaics must be very slight, if it exists at all.

This fact is significant. For if one makes a general "physiological Minute condition" responsible for the occurrences of spot-producing processes one might expect to find a corresponding seriation of different Minutes in respect to their potencies to produce both sex-linked and autosomal spots. However, a seriation in respect to frequency of sex-linked mosaics would show roughly:

$$\text{Blond-Minute} > Mn > M33j,\ Mw,\ My$$

while the same Minutes (*Mn* excluded) with respect to frequency of autosomal mosaics probably would have to be arranged as:

$$Mw,\ My > M33j,\ \text{Blond-Minute}$$

The two seriations are given after an analysis of the data presented in different tables of this paper. Too great reliance cannot be attributed to details of these arrangements, as the experiments were carried out over a period of years and under different genetic and environmental conditions. Therefore no special table has been made up from these data, as the quantitative results might indicate a higher degree of accuracy than they really represent. However, there seems no doubt as to the validity of the main result, namely that the effect of Minutes on the frequencies of sex-linked and of autosomal spots varies independently so that one Minute factor may affect strongly the number of sex-linked but only slightly the number of autosomal spots and vice versa.

An even more striking correlation between certain autosomal Minutes and areas mosaic for definite regions of the same autosome will be presented in the chapter on "Autosomal spots."

THE MECHANISM OF MOSAIC FORMATION

Various hypotheses

In the foregoing pages the effect of Minutes on the process of mosaic formation was discussed in general terms. The following part will contain an analysis of the process itself.

BRIDGES' work with females carrying *Mn* in one X and recessive genes in the other seemed to have established (1) that the cells of a spot do not

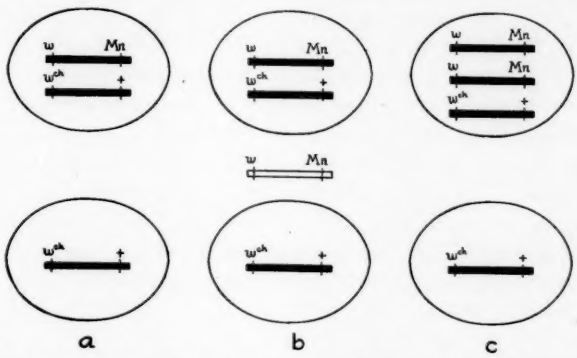


FIGURE 1 a–c. Three possibilities to account for elimination of an X chromosome.

contain the *Mn* chromosome and (2) that these cells are male in constitution, containing only one X with the recessive genes. Three main possibilities suggested themselves as mechanisms for the elimination of the *Mn* chromosome from the cells of the spot: (a) during a somatic division the *Mn* X chromosome does not divide and consequently passes into one daughter nucleus, leaving the other one in possession of only a division product of the not-*Mn* X chromosome; (b) the *Mn* X chromosome divides into two halves, but only one half passes into one of the daughter nuclei. The other half lags behind, is not included into a daughter nucleus, and degenerates in the cytoplasm; (c) the *Mn* X chromosome divides into two halves. These halves do not disjoin but pass together into one daughter nucleus.

The constitution of the daughter nuclei according to the three hypotheses is pictured in figure 1. No way of distinguishing between the mechanisms (a) and (b) was found, but a test between (a) and (b) on one side and (c) on the other seemed possible. The sister cell of the "elimination

cell" in (a) and (b) would be of the same constitution as the original constitution of the fly, while in (c) it would have a different constitution, being triplo-X. By using suitable gene markers such a condition as (c) could be demonstrated if it occurred. Accordingly females were bred which had white in their *Mn* chromosome and cherry (w^{ch}) in the other X chromosome. The eye color in such females is a light cherry. Their eyes were searched for spots of normal, dark cherry color, indicating the elimination of the *w Mn* chromosome. When such spots were found it was determined whether their surroundings in the eye were of the w/w^{ch} coloration as expected according to (a) and (b) or whether they contained a very light cherry spot, indicating the $w/w/w^{ch}$ constitution expected according to (c). On 1702 w *Mn*$/w^{ch}$ females which were inspected 20 eye spots were found. They all were cherry colored and unaccompanied by a twin spot of $w/w/w^{ch}$ coloration or of the irregular facet arrangement characteristic for 3X+2A eyes. Sixteen of these spots covered an area of at least 3 or 4 but not more than about 50 facets; 4 spots included more than 150 and less than 300 facets. These facet numbers have not been determined accurately but have been estimated from sketches. The total number of facets in one eye is between 700 and 800 in females. Similarly, in 168 w^{ch}*Mn*$/w$ females, two white eye spots were observed not accompanied by a twin spot of the darker colored triplo-X constitution w^{ch}*Mn*$/w^{ch}$*Mn*$/w$. These results rule against the hypothesis (c) provided that cells with two *Mn* factors and one normal allele are viable and can give rise to cell patches large enough to be recognized as mosaic spots. Nothing was known originally about this point.

Somatic segregation

y/sn^3 flies; preliminary discussion

The solution was brought about by chance. When it had been found that the "Minute condition" caused by an "uncovered" Blond-deficiency was necessary for the elimination of the deficiency X chromosome, an experiment was made in order to determine whether a deficiency which in itself does not produce a Minute effect would, together with an independent Minute factor, bring about the elimination of the deficient chromosome. The deficiency chosen was the well known Notch-8 (N^8) in the X chromosome and the Minute was the autosomal *Mw*. The following cross was made: N^8/y *Hw* dl-49 ♀ by sn^3; *Mw*/+ ♂, and the frequencies of head-thorax spots in N^8 and not-N^8 sister females were compared (table 4). The presence of *Hw* and of the dl-49 inversion is irrelevant. From 280 $N^8/sn^3;+^M$ or *Mw* females 9 mosaic spots were obtained while 381 control y *Hw*/dl-49/$sn^3;+^M$ or *Mw* females yielded 15 spots. Thus the frequency

of spots in N^8 was 3.6 per cent, in controls 3.9 per cent. There was no interaction of the Notch deficiency with the autosomal Minute-w.

The nine spots in the N^8/sn^3 flies were recognized by singed setae occurring presumably as a consequence of elimination of the N^8 chromosome. In cases where the sn^3 containing X chromosome would have been eliminated, no visible spot would have been produced, for the N^8 chromosome contained no recessive genetic marker which would have expressed itself in a spot (provided that a cell containing only the Notch-deficient X chromosome is able to reproduce sufficiently to give rise to a large enough cell-patch).

TABLE 4

N^8/y Hw dl-49 by sn^3; Mw/+. Head and thorax spots only.

	N^8		$+^N$	
	$+^M$	*Mw*	$+^M$	*Mw*
y spots	—	—	1	1
sn^3 spots	4	5	0	2
$y-$ / sn^3 twin spots	—	—	2	9
Total spots	**4**	**5**	**3**	**12**

The situation was different in the *y Hw* dl-49/sn^3 control flies. If there was no preference which of the two X chromosomes would be eliminated, then two different types of spots might be expected to occur in about equal numbers: (1) *y* spots in case of elimination of the sn^3 X chromosome and (2) sn^3 spots in case of elimination of the *y Hw* dl-49 X chromosome. The 15 spots found consisted of (1) two *y* spots and (2) two sn^3 spots while (3) the remaining 11 spots showed an unexpected structure: *they were twin-spots formed by a yellow not-singed area adjacent to a singed not-yellow area.*

The obvious explanation is that, during a somatic division of one of the cells of these 11 *y Hw* dl-49/sn^3 females, a segregation had taken place whereby one daughter cell obtained the yellow gene carried originally by one of the X chromosomes while the other cell obtained the singed gene carried originally by the other X. A process similar to gametic segregation of genes lying in opposite members of a pair of chromosomes had occurred in a somatic cell. The further division and normal somatic differentiation of the two daughter cells finally gave rise to mosaic twin areas.

These findings of somatic segregation suggested that the so-called chromosome elimination in certain cells leading to the appearance of mosaic spots was in all or most cases the consequence of somatic segrega-

tion. This theory is substantiated by three facts. (1) Further experiments demonstrated the general occurrence of twin spots in flies of suitable constitutions. (2) In appropriate experiments it could be shown that nearly all mosaic spots exhibit the results of somatic crossing over. This makes simple elimination hypotheses improbable. (3) The theory solves the difficulties encountered by the assumption that the sex-linked Minutes eliminate only their own chromosomes.

We shall first discuss point (3). The more important statements (1) and (2) will be dealt with in later sections.

Minute-n and Blond-Minute "elimination" as somatic segregation

Somatic segregation of the X chromosomes in a female carrying *Mn* in one X and a recessive gene in the other will lead to two daughter cells, one containing only *Mn*, the other only the recessive. *Mn* is known to be lethal to a male or a homozygous *Mn* female zygote. If we assume *Mn* in such a condition to be lethal to a somatic cell also we shall expect the one daughter cell to die while the other one, containing only the recessive, will give rise to the observed spot. (It might be argued that a cell containing only *Mn* is viable but phenotypically not different from the non-segregated surrounding tissue. This, however, is excluded by having a recessive gene together with *Mn* in the one X chromosome but not in the other X. Segregation without lethal effect of the *Mn* segregation product should exhibit *Mn* spots which also show the recessive gene effect. In the experiments discussed on p. 635 the *Mn* chromosome contained a white or cherry gene, but no spots showing the respective eye colors were found. Other experiments of similar nature are described in later sections of this paper.)

Somatic segregation can account also for the single spots in "Bld-Minute" flies. In heterozygous Bld-Minute females it will lead to a "not Bld-Minute" and a "Bld-Minute" cell. The former will give rise to a spot with not "Bld-Minute" phenotype while the latter is expected to die, that is, if one assumes a cell not to be viable in case it contains a completely uncovered X chromosome deficiency. Male zygotes containing the uncovered Bld-deficiency X chromosome or female zygotes homozygous for such a condition are known to be not viable.

The reader might be inclined to strengthen these arguments by pointing to DEMEREC's studies (1934) on cell lethals. This, however, would not be justified as DEMEREC's interpretation is *based* on the acceptance of the theory of somatic segregation and therefore cannot be used to prove this theory.

The influence of a Minute condition on the occurrence of mosaic spots thus consists in an increase of the tendency to somatic segregation. The

question asked at the close of the chapter on mosaic spots in Bld-Minute flies can now be answered. "Is there an interaction of such a kind that 'the physiological Minute condition' produced by an uncovered deficiency in the X chromosome would eliminate just this deficient chromosome?" The answer is this: elimination is not restricted to one X chromosome, but somatic segregation of the two X chromosomes leads to the appearance of spots which possess the non-deficient chromosome only.

Further analysis of somatic segregation in y/sn^3 flies

In order to justify the assumption of somatic segregation as a general cause of the occurrence of mosaic spots we have here to deal with those cases of table 4 which did not show a twin condition although such a condition might have been expected, for whenever a cell contains in each of its two X chromosomes one or more recessive, heterozygous genes capable of producing their phenotypes in small surface spots, the simple process of segregation will yield two neighboring cells pure for the genes of their respective segregated chromosomes. Why then did only 11 out of 15 cases show the twin spot condition? The following considerations have to enter into an answer to this question. (a) Even under the assumption that somatic segregation always leads to two sister cells which are both pure for originally heterozygous recessive genes, the appearance of the mosaic region will depend on the time of the segregation process in ontogeny. With a very late occurrence in development only two small twin areas could be produced, each consisting of only one cell if segregation took place during the very last division of the cells of the imaginal disc. In such cases it will frequently happen that one of the segregation products will form a seta and thus be recognized by its singed shape or yellow color, while the other segregation product will not happen to build up such a part of the hypodermis as will give rise to a seta and thus will not be recognizable. In other words, small twin spots will be liable to be recognized only in one of the segregation products. In large spots the probability is correspondingly higher that the areas formed by both segregation products will contain setae. They should therefore show the twin condition. (b) Even in larger spots a twin condition will be found lacking in case the cell descendants of one of the segregation products happen to become located in a region bare of setae, as for instance most of the scutellum, large areas on the head, the sternopleurae, the regions laterally from the posterior dorsocentrals. Twinning will be absent also in cases where the survival or reproductive ability of one of the daughter cells of the segregating division has been impaired, be it by chance, by normal developmental determination, or by lower genetic viability. To sum up the preliminary discussion: Small spots are expected to be often "singles," not twins; large spots in the majority of cases should be twins.

Tables 5 and 6 contain data pertinent to this question. Four different but fundamentally similar groups of experiments are summarized. In experiment 1 the females were of the constitution *y Hw* dl-49/*sn*^{3} with or without *Mw*. They include, together with others, the flies discussed at the beginning of the present chapter. It is seen (table 5) that 23 out of 38

TABLE 5

Kinds and sizes of spots in experiments involving primarily y and sn^3 *when located in opposite chromosomes.*

EXP.	CONSTITUTION	INDIVID.	SPOTS	*y* SPOTS			*sn*^{3} SPOTS			*y-sn*^{3} TWIN SPOTS	
				NO. OF SETAE			NO. OF SETAE			NO. OF SETAE	
				1	2	>2	1	2	>2	2	>2
(1)	*y Hw* dl-49/*sn*^{3}	551	38†	2	—	2	8	1	2	4	19
(2)	*y w* (or w^e)/*sn*^{3}	376	212‡	40	9	12	62	12	10	7	60
(3)	*y g*^{2}*bb*/*sn*^{3}*bb*^{z} (a)	635	6†	—	—	1	5	—	—	—	—
	(b)	635	157*	22	8	6	60	14	9	9	29
(4)	*y bi cv ct*^{6} *v* *g*^{2} *bb*^{1}/*sn*^{3}*bb*^{z} (a)	214	19†	1	—	—	5	4	8	—	1
	(b)	214	73*	16	4	1	12	7	15	3	15
				81	21	22	152	38	44	23	124
	Totals	**1776**	**505**	**124**			**234**			**147**	

† Head and thorax spots only.
‡ Head, thorax and abdominal spots.
* Abdominal spots only.

spots were twins and that 10 out of the 15 single spots were so small as to include only one seta, which obviously makes it impossible for them to show a twin condition. Of the 28 spots covering two or more setae the great majority, namely 23, were twins. Experiment 1 then corresponds closely to our expectation. In experiment 2 the constitution of the flies was *y w*/*sn*^{3} or *y* w^e/*sn*^{3} (*w* and w^e will be disregarded here). In addition, all individuals contained *M33j*. There were 67 twin spots out of a total of 212, and 102 out of the 145 not-twin spots were single seta spots. Of the 110 spots covering two or more setae 67 exhibited the twin condition, and 43 did not. But 21 of these 43 were so small as to include only two setae, thus still making it probable that the supposed twin area did not happen to cover a setae-forming region. Although the results in experiment 2 did not come as near to expectation as in 1 the agreement can be regarded as sufficient. The results will be further discussed after a description of experiments 3 and 4. In these a high frequency of spotting was induced by making the flies homozygous for recessive, mutant alleles of bobbed. In 3 one allele was the standard *bb*, the other one either the same or a very similar one; in 4 one was the lethal allele *bb*^{1} the other was as in 3. Bobbed

can be called a recessive Minute gene, so that the flies in these experiments were under the influence of a "physiological Minute condition," which caused the high spotting frequency. Again the presence of other genes besides *y* and *sn*^{3} will be disregarded at this point. In 3 only 38 out of 163 spots were twins, but a consideration of the different sizes of spots again shows that 87 out of the 125 single spots were so small as to include only one seta. Of the remaining 38 single spots, 22 were so small as to include only two setae, but of the 76 spots covering two or more setae, there were 38 twin spots. Experiment 3 then, although showing a general agreement with expectation seems to deviate more from it than the two experiments 1 and 2. Before discussing this we shall consider experiment 4. Here 19 out of 92 spots were twins, and 34 out of the 73 single spots included only one seta. Of the remaining 39 single spots, 15 were so small as to include only two setae. Out of the total of 58 spots covering two or more setae, 19 exhibited the twin condition. As in experiment 3 these results seem to be in general, but not very close, agreement with the theory of simple somatic segregation as the cause of spotting.

TABLE 6

Further data on the size of spots in experiments 1–4 of table 5.

	(1)	(2)	(3)	(4)
Total *y* setae in single spots	137	182	69	26
Total *sn*^{3} setae in single spots	113	145	121	183
Total *y* setae in twins	121	230	64	27
Total *sn*^{3} setae in twins	80	208	86	45
Average *y* setae in twins	5.3	3.4	1.7	1.4
Average *sn*^{3} setae in twins	3.5	3.1	2.3	2.4
Average (*y*+*sn*^{3}) setae in twins	8.8	6.5	4.0	3.8

Table 6 summarizes certain facts which help to explain the apparent discrepancies. The last horizontal line shows that the average size of the twin spots in experiment 1 was more than double that of the twin spots in 3 and 4 while it was intermediate in 2, and that in 3 and 4 the average size of one of the twin areas was only of the order of magnitude of two setae. It follows that there was a considerably higher chance for the small twin areas in 3 and 4 to appear phenotypically only as single spots than there was for the large areas in 1 and 2. Part of the deviations from our expectation are further cleared up by the following considerations. If single spots are really parts of twin areas in which only one area had the opportunity to exhibit its phenotype, then if chance alone determined which of the two areas covered a seta-forming region one should expect an equal frequency of *sn*^{3} and of *y* single spots. An inspection of table 5, however, shows that,

at least in 3 and 4, there is no 1:1 ratio of the two types of spots. In different form this can be seen from the first two lines of table 6 where the total

TABLE 7

List of all 124 twin spots >2 from table 5 with number of y and sn³ setae affected. Each horizontal line is the record of one kind of spot. The numbers in parentheses indicate the frequency with which this type of spot was represented. No number in parentheses was given when there was only one spot of its kind.

EXP. (CF. TABLE 5)	NO. OF SETAE y	NO. OF SETAE sn^3	DIFFERENCE: NO. y–NO. sn^3
(1)	3	1	+ 2
	28	1	+27
	(2) 1	2	− 1
	(3) 3	2	+ 1
	4	2	+ 2
	(2) 1	3	− 2
	3	3	0
	4	3	+ 1
	24	3	+21
	5	4	+ 1
	3	5	− 2
	5	5	0
	5	9	− 4
	1	14	−13
(2)	(7) 2	1	+ 1
	(2) 3	1	+ 2
	(2) 4	1	+ 3
	5	1	+ 4
	10	1	+ 9
	50	1	+49
	(4) 1	2	− 1
	(4) 2	2	0
	(3) 3	2	+ 1
	5	2	+ 3
	(5) 1	3	− 2
	(3) 2	3	− 1
	(2) 3	3	0
	4	3	+ 1
	(2) 5	3	+ 2
	(2) 6	3	+ 3
	(4) 1	4	− 3
	2	4	− 2
	(3) 3	4	− 1
(2) (cont'd)	(2) 1	5	− 4
	2	5	− 3
	4	5	− 1
	6	6	0
	1	7	− 6
	15	7	+ 8
	3	7	− 4
	1	10	− 9
	11	14	− 3
	1	15	−14
(3)	(5) 2	1	+ 1
	5	1	+ 4
	(6) 1	2	− 1
	(2) 2	2	0
	(2) 3	2	+ 1
	1	3	− 2
	(2) 2	3	− 1
	1	4	− 3
	(2) 2	4	− 2
	3	4	− 1
	4	4	0
	(2) 1	5	− 4
	2	5	− 3
	3	7	− 4
(4)	(2) 2	1	+ 1
	3	1	+ 2
	(5) 1	2	− 1
	2	2	0
	(3) 1	3	− 2
	3	3	0
	1	4	− 3
	2	5	− 3
	1	6	− 5

number of *y* and sn^3 setae has been noted. It is evident that the *y* setae are in the minority in 3 and 4, while there is a deficiency of sn^3 setae in 1 and especially in 2. Possible causes for these new discrepancies can be learned

from the second part of table 6. Here the frequencies of *sn*^{3} and *y* setae are listed for all twin spots, that is, for all cases in which there is no doubt as to the occurrence of somatic segregation. The relative frequencies of the two types of setae in twin spots vary in the same direction as the total frequencies in single spots. Obviously, in these experiments, the chances for survival and reproduction of the two daughter cells from a segregating division were not equal. The vitality of *y* cells was lower than that of *sn*^{3} cells in 3 and 4 but higher in 1 and 2. The greater the inequality in survival value, the higher the proportion of spots even of larger sizes which should appear only as single spots, the twin area having died or been kept small. This is roughly borne out by the data. What the causes of lowered viability were in these experiments cannot be determined accurately now. The presence of different alleles of bobbed in the two X chromosomes of 4 and possibly 3 has nothing to do with it, as will be shown later. However, it is suggestive that the cells in 4 which are pure for *y* are also pure for *bi*, *ct*^{6}, *cv*, *v*, and *g*^{2}. As it is known that this multiple mutant condition considerably lowers the viability of a whole individual, the assumption seems justified that a similar effect may be found also in mosaic parts.

Table 7 has been added to give a more complete representation of all twin spots with more than 2 setae which occurred in these experiments. It is interesting that such great inequalities of the two twin areas were observed as the case in which *y* area covers 50; *sn*^{3} area covers 1; or *y* area has 24 setae and *sn*^{3} area 3; or *y* area 1 and *sn*^{3} area 15 seta.

Some of these very unequal twin spots might possibly be regarded as two single spots of independent origin, lying next to each other, but this must be very exceptional. The frequency of spots in most experiments is low enough as to make rare the occurrence of more than one spot on an individual, although the incidence of flies with two or more spots is higher than according to chance (BRIDGES in MORGAN, STURTEVANT and BRIDGES 1929; also numerous data of the author). If several spots occur on one fly they have no tendency to be neighbors.

Summing up, it seems clear that somatic segregation does not only account for the occurrence of twin spots but also for that of single spots. But while the evidence in the cases of twin spots is direct, in the cases of single spots it is of such a nature as to leave open the possibility that not all single spots can be regarded as vestigial twin spots whose region did not happen to affect a seta. A numerical treatment of the data which theoretically should be able to give a final decision is not feasible on account of the many variable factors involved. The next section, however, will show that a certain proportion of single spots are to be expected which have never been partners of an original phenotypic twin group.

Somatic segregation and crossing over
Experiments involving *y sn*^{3}/+ flies

In the experiments summarized in table 5, *y* and *sn*^{3} were in opposite chromosomes. When both mutants were in the same chromosome, some new results were obtained (table 8): Three kinds of spots appeared with

TABLE 8

Spots in flies of the basic constitution y sn^3/+.

EXP.	CONST.	IND.	SPOTS	*y sn*^{3}			*y*			*sn*^{3}			OTHER SPOTS
				1	2	>2	1	2	>2	1	2	>2	
(1)	*y sn*^{3}*bb/car bb*	83	34	10	4	6	3	3	5	2	—	1	—
(2)	*y sn*^{3}*bb/car bb*	495	72	25	15	10	12	2	5	2	—	1	—
(3)	*y sn*^{3}*bb*/+†	508	35	10	11	4	5	4	—	1	—	—	—
(4)	*y sn*^{3}/+†	321	21	8	3	4	1	1	2	—	—	—	2*
				53	33	24	21	10	12	5	—	2	
Totals		**1407**	**162**		**110**			**43**			**7**		**2**

† Partly *Mw*/+.

* $+^{v}+^{sn}$; ♂-colored.

different frequencies, namely 110 *y sn*^{3}, 43 *y* and 7 *sn*^{3} spots. The finding of *y sn*^{3} spots was expected, for segregation in *y sn*^{3}/+ females (disregarding the presence of *bb* and *car*) should give rise to *y sn*^{3} and + cells, which would be visible as *y sn*^{3} spots. The occurrence of *y* and of *sn*^{3} spots needs an additional interpretation. Somatic crossing over between *y* and *sn*^{3} would separate these two genes from each other and thus afford an explanation. If the crossover process occurred during a two strand stage, the resulting strands would be $y+^{sn}$ and $+^{v}sn^{3}$, and if segregation ensued a *y* and a *sn*^{3} twin spot would be produced. No twin spots were found, making the two strand crossing over assumption invalid. If, however, somatic crossing over occurred at a four strand stage between two of the four strands, and segregation two strands by two strands followed, then the facts can be explained (fig. 2). It is seen that following single crossing over, different types of chromatid segregation, namely **x** and **y,** give rise to either *y* or *sn*^{3} single spots. (Throughout this paper the term "chromatid" is used in reference to the strands which *constitute* a multivalent chromosome group during prophase and metaphase, as well as in reference to those chromosomes of anaphase and telophase which *originated* from a multivalent.)

If crossing over occurs at the four strand stage the subsequent segregation process will be expected to lead to either one of two results. (1) A separation of the four chromatids into two daughter cells will occur, followed

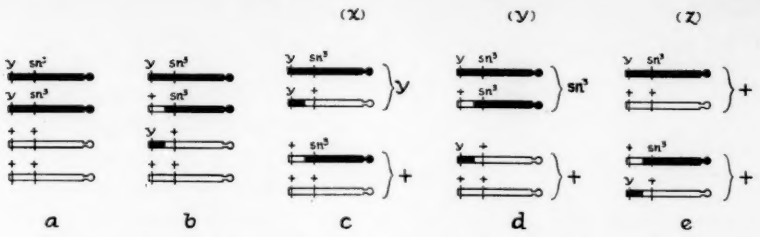


FIGURE 2. $y\ sn^3/+$. Crossing over between y and sn^3 at a four strand stage. a. Non-crossover chromatids. b. Two crossover and two non-crossover chromatids. c-e. Three different types of chromatid segregation.

later by normal mitosis or (2) the initiated segregation will bring about a true reduction of chromatids leading to a second segregating division with resulting cells containing only single X chromatids. In females of the constitution $y\ sn^3/++$, such a reduction process, after crossing over between two of four strands, should lead to four cells with the genes $y\ sn^3$, $y+^{sn}$, $+^{y}sn^3$ and $++$. The areas resulting from later cell-divisions of the segregation products would exhibit the phenotypes $y\ sn^3$, y, and sn^3. The visible result would be a triple spot. Not a single spot of this nature was observed; the hypothesis of a complete somatic reduction process is thus refuted.

We can test the assumption of somatic crossing over at a four strand stage by applying it to the earlier experiments in which the constitution of the flies was y/sn^3. Single crossing over between y and sn^3 at a two strand stage would yield $y\ sn^3$ and $++$ strands; segregation would result in $y\ sn^3$ spots. No such spots have been found (table 5). Crossing over at a four strand stage, however, would, according to different types of chromatid segregation, produce y or sn^3 single spots (fig. 3). Such spots did occur and while many of them could be regarded as vestiges of potential twin spots, it is possible to assume a certain number of them to have been products of crossing over between y and sn^3 in the manner suggested.

Again it is obvious that no reduction of chromatids occurred which

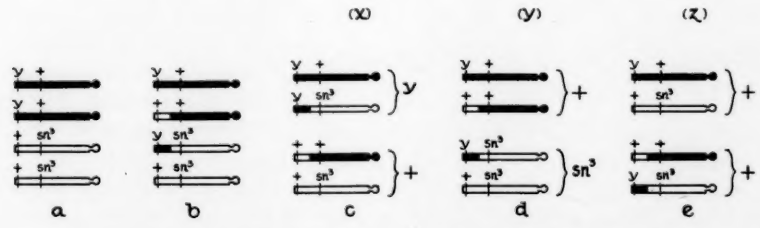
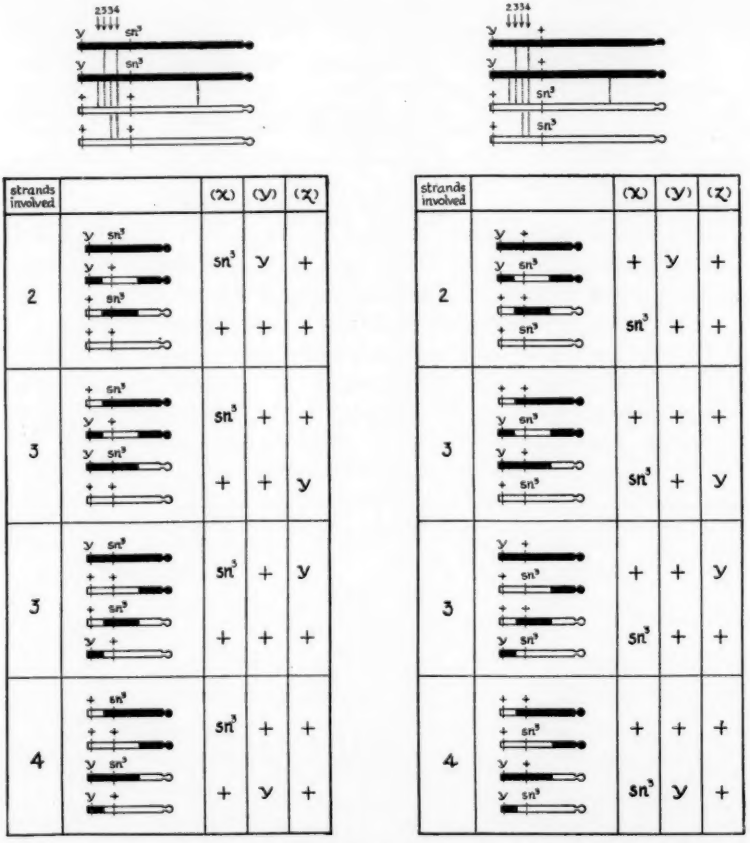


FIGURE 3. y/sn^3. Crossing over between y and sn^3 at a four strand stage.

would have resulted in triple spots *y*, *sn*^{3}, and *y sn*^{3}. No triple spots were found and the phenotype *y sn*^{3} did not occur even in single spots.

If one characterizes the two types of chromatid segregation **x** and **y** by the constitution of the right chromatid ends, then **x**-segregation would be

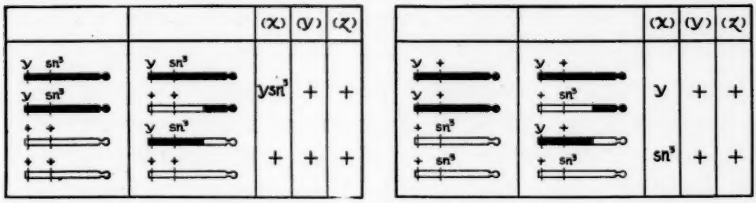


FIGURES 4 (LEFT) AND 5 (RIGHT). *y sn*^{3}/+ and *y*/*sn*^{3}. The four different types of double crossovers, involving 2, 3, and 4 chromatids and the results of the three different types of segregation.

equational and **y**-segregation reductional. As the right end of the X chromosome is known to contain the fibre attachment point, processes **x** and **z** would result from separation of sister attachment points while **y** would occur only when sister points stay together. It is possible to account for all observed spots with the assumption of equational segregation for the

right end, if one considers the possibility of occurrence of somatic double crossovers simultaneously to the left and to the right of sn^3 (figs. 4, 5). Four different kinds of double crossover processes within the four strand "tetrad" are possible, involving two, three, and all four strands. After single crossing over **x**-segregation results in *y* single spots in flies of both constitutions *y* $sn^3/++$ and $y+/+sn^3$, after double crossing over it results in sn^3 spots. If **y** segregation occurs, it gives rise to *y* spots in one-half of all cases; similarly **z** segregation produces visible *y* spots in two out of the four cases.

While a decision between the hypotheses of single crossing over and different kinds of segregation or single and double crossing over and equa-



FIGURES 6 (LEFT) AND 7 (RIGHT). *y* $sn^3/+$ and y/sn^3. Results of crossing over to the right of sn^3.

tional **x**- and **z**-segregation only cannot be derived from the present data, the second hypothesis seems to be in better agreement with the known facts in regard to the separation of daughter chromosomes in mitosis. We shall return to this question in the next chapter.

What is the frequency of the different crossover types in the *y* $sn^3/+$ experiments? We shall regard (and shall justify this hypothesis later) the *y* sn^3 spots as due to single crossing over between sn^3 and the right end of the X chromosome (fig. 6; see also fig. 7 for the y/sn^3 flies). The sn^3 spots are considered to be due to double crossing over. In determining the frequency of crossing over we cannot follow the usual procedure in germinal crossing over and base the calculation on the total of observed non-crossovers and crossovers, since the absence of crossing over in the great majority of somatic cells makes the number of non-crossovers rather meaningless. However, we can determine the relative frequencies of observed crossovers to the right and to the left of sn^3 and the frequency of double crossovers. The frequencies of *y* sn^3, *y*, and sn^3 spots were 110, 43, and 7. As was shown earlier, some *y* spots have to be regarded as products of **z**-segregation after double crossing over. As they constitute one-third of all visible double crossover products, their number is taken as 3.5. After subtracting this number from the total number of *y* spots, we find the proportion of spots produced after crossing over to the right of sn^3 (*y* sn^3 spots)

to spots from crossing over to the left of sn^3 (y spots) to be 110:39.5. Only the **x**-type of segregation in single crossing over yields visible spots, while the equally frequent **z**-type remains undetected. In double crossing over both **x**- and **z**-segregations result in visible spots, a total of 75 per cent of all cases. In order to have comparable figures we have to insert two-thirds of the number of visible double crossover spots, namely $(7+3.5).\frac{2}{3}=7$. Thus the proportion, single crossovers to the right of sn^3:singles to the left:doubles is 110:39.5:7. Comparing these values with the corresponding frequencies in meiosis, we find for the single crossovers 36:20.5. (MORGAN, BRIDGES, and STURTEVANT 1925, modified; no satisfactory data are available for the double crossovers.) In mitosis there seems to occur a higher proportion of all crossovers to the right of sn^3 than in meiosis. Even this frequency of somatic crossovers to the left of sn^3 was unusually high, as further experiments show.

Experiments involving Blond

In table 9 experiments are summarized in which somatic crossing over in females heterozygous for Blond, and either sn^3 or a forked (f, 56.7) allele was studied. The flies had two normal chromosomes II and were therefore of Minute type on account of the Blond deficiency in the X chromosome. In experiment (1) 167 out of 179 spots can be explained as

TABLE 9

Spots in Blond-Minute flies heterozygous for sn^3, f, or f^6; mainly head-thorax spots except in exp. (2).

EXP.	CONST.	SPOTS	TYPE OF SPOT*		
			$+^{Bld}+^{m}+^{M}$	$+^{Bld}m+^{M}$	$+++-+f^6+$
(1)	$\frac{Bld\ +}{+\ sn^3}$	179	9(+3†)	167	—
(2)	$\frac{Bld\ +}{+\ f^6}$	178	8	168	2
(3)	$\frac{Bld\ sn^3}{+\ +}$	91	91	—	—
(4)	$\frac{Bld\ f}{+\ +}$	15	15	—	—

* m stands for either sn^3 or f; $+^M$ for "not Bld-Minute," i.e., long setae; "–" signifies a twin spot condition (+++ next to $+f^6+$).

† The three spots had $+^{Bld}+^{sn}$ setae of still smaller length than Bld-Minute.

products of crossing over to the right of *sn*^{3}, for if such crossing over occurs, normal equational disjunction of daughter fibre points will produce two sister cells of the constitution Bld+/Bld+ and +*sn*^{3}/+*sn*^{3}. The former is assumed not to be able to give rise to a viable patch of cells, the latter multiplies to produce a $+^{Bld}$ *sn*^{3} spot with normal sized setae. As can be readily seen from constructing a diagram for this case similar to our figure 7 for a previously discussed experiment, the other two mathematically possible types of disjunctions **y** and **z**, do not give rise to visibly new constitutions. However, crossing over to the left of *sn*^{3} and subsequent disjunction of sister attachment points (fig. 3) can lead to sister cells Bld+/Bld *sn*^{3} and ++/+ *sn*^{3} the latter of which result in normal sized, not-Bld, not-*sn*^{3} bristles. Nine such spots were found. Non-disjunctional segregation **y** of the fibre points would produce Bld *sn*^{3}/+*sn*^{3} bristles. No such case was found. Double crossovers which after normal disjunction of the fibre points (similar to figure 5) also would yield Bld *sn*^{3}/+*sn*^{3} spots besides half as many wild type spots were also absent. (There were three spots with not-Bld, not-*sn*^{3}, but shorter than Bld-Minute bristles. Their nature is not clear.)

In summing up experiment 1, we find the great majority of spots 167 out of 179, to be due to crossing over to the right and only 9 due to crossing over to the left of *sn*^{3}. This is a much lower proportion of left crossovers than in germinal crossing over and also considerably lower than in the experiment with *y sn*^{3}. That no case of double crossing over was encountered agrees with the rarity of left crossovers. A nearly identical result was obtained in experiment 2 which was of a similar nature as 1 except for the use of *f*^{5} instead of *sn*^{3}. In spite of *f*^{5} being 35.7 map units farther away from the left end than *sn*^{3} the proportion of left to right crossovers was still 8:168, disregarding the two twin spots in the last column. Again no double crossover types occurred. (One of the two twin spots covered the whole left mesothorax except the scutellum with a division line posteriorly from the anterior notopleural, presutural and anterior dorsocentral bristles which separated the anterior +^{f} part from the posterior *f*^{5} part; the other not quite decisive spot was formed by a left +^{f} ant. postalar and *f*^{5} ant. scutellar bristle. These spots can formally be explained by two crossover processes, first one to the left of *f*^{5}, resulting in a +/*f*^{5} cell, and a second one occurring in this cell or one of its near descendants and segregating +/+ and *f*^{5}/*f*^{5} twins.)

Summing up experiments 1 and 2, it is seen that the relative frequency of left crossovers is very low. Especially interesting in view of the occurrence of 17 cases of left crossovers with normal disjunction of the fibre points is the comp ete absence of left crossover cases with non-disjunction of the fibre points. As pointed out before, they would have been visible

TABLE 10*

Spots in flies containing y sn^3 and Mn.

In exp. (2) some of the individuals were of the constitution XXY (see p. 709).

In exp. (3) the not-Mn X chromosome carried besides y, the genes g^2 and ty (tiny bristles). The presence of the latter generally made a separation of Mn and $+^{Mn}$ spots impossible.

EXP.	CONST.	IND.	SPOTS	% SPOTS	+			y sn^3			y			sn^3			y-sn^3		+ ♂ COLOR
					1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
(1)	$\frac{+\ sn^3\ Mn}{+\ +\ +}$	1038	29	2.8	5	2	8	3	—	—	3	2	1	3	—	—	—	—	2
	Totals						**15**			**3**			**6**			**3**			**2**
(2)	$\frac{y\ +\ Mn}{+\ sn^3\ +}$	349	254	72.8	3	—	1	—	—	—	33	6	1	60	23	112	3	4	8
	Totals						**4**						**40**			**195**		**7**	**8**
(3)	$\frac{+\ sn^3\ Mn}{y\ +\ +}$	1040	121	11.6	—	—	—	—	—	—	25	9	69	11	1	3	1	2	—
	Totals												**103**			**15**		**3**	
(4)	$\frac{+\ +\ Mn}{y\ sn^3\ +}$	827	96	11.6	—	—	—	25	13	51	4	1	—	2	—	—	—	—	—
	Totals									**90**			**5**			**2**			

* In exp. (1), the 3 y sn^3 spots and 2 of the sn^3 spots were probably *Mn*.
In exp. (2), 4 *y* spots were probably *Mn*, 103 sn^3 spots were $+^M$, and one sn^3 spot had very small setae.
In exp. (3), 10 sn^3 spots were probably *Mn*.
In exp. (4), 33 *y* sn^3 spots were $+^M$, one was probably *Mn*. The total of 90 *y* sn^3 spots includes one of unrecorded area. Of the *y* and the sn^3 spots one had small setae and one setae similar to $+^M$.

spots with Bld sn^3 (or Bld f^5) setae. Their absence shows that non-disjunction of the daughter fibre at least in these cases of left crossing over either does not occur at all or is rare.

In experiments 3 and 4 only one type of spot was found. This type corresponds in origin to most of the spots in 1 and 2 namely either to the single crossovers to the right or to the left crossovers, both with normal disjunction of the daughter fibre points. Each of these types gives rise to $++$ spots. Bld sn^3 (and $++$) spots would have been produced if single crossing over and non-disjunction of daughter fibres or if double crossovers had occurred. The absence of such spots agrees with the absence of corresponding spots in 1 and 2.

Finally, it is easily seen that no chromatid reduction has taken place, as certain types of twin spots should have occurred in all four experiments with Blond. Their absence is proof of segregation, two strands by two strands, without reduction.

Experiments involving *y*, sn^3, and *Mn*

The finding of somatic crossing over along the length of the X chromosome seems to contradict BRIDGES' (1925) results with spots in Minute-n flies. He states that no spots occurred which showed recessive characters determined by genes located in the *Mn*-carrying chromosome nor that spots occurred which showed only some, but not all, characters determined by genes in the other X. In the language of the crossing over theory this would mean no somatic crossing over occurred along the main length of the chromosomes, from *y* (0.0) to *Mn* (62.7). In order to clear up the apparent discrepancy, flies heterozygous for *y*, sn^3, and *Mn* were produced and scrutinized for spots. All four possible different distributions of these three factors and their alleles over the two X chromosomes were investigated. The results are assembled in table 10. In most spots no distinction could be made between *M* and $+^M$ seta length. Wherever this character could be classified it is recorded in the footnote. It is apparent that in each of the four experiments more than a single type of spot was found. However, in each case one type predominates. In the only case in which this prevalence amounts to less than 75 percent of all spots an external cause can be seen to be responsible, namely the most frequent class of spots in experiment 1 is distinguished from the surrounding tissue by its $+^M$ condition only. Spots of this constitution are recognizable, in general, only if they include one or more macrochaetae on the head or thorax, while all other spots exhibiting *y* or sn^3 or both *y* and sn^3 are recognizable anywhere and both in macro- and microchaetae. If only those spots are tabulated which have about equal chances of being recognized, namely head-thorax spots which include macrochaetae, their numbers are found to be

TABLE 11

Results of **x** *(equational) and* **y** *(reductional) segregation in experiments involving y, sn^3, and Mn. In each case the upper symbols represent one segregate and the lower symbols represent its twin segregate.*

EXP.	SINGLE CROSSOVER IN REGION	SEGREGATION* (x)	(y)
(1)	1	*y Mn* *Mn*	dies +
$\frac{y\ sn^3\ Mn}{+\ +\ +}$	2	*y* sn^3 *Mn* *Mn*	dies +
	3	dies +	*Mn* *Mn*
(2)	1	*y Mn* *Mn*	dies sn^3
$\frac{y\ +\ Mn}{+\ sn^3\ +}$	2	*y Mn* sn^3 *Mn*	dies +
		dies sn^3	*Mn* *Mn*
(3)	1	*Mn* *y Mn*	dies +
$\frac{+\ sn^3\ Mn}{y\ +\ +}$	2	sn^3 *Mn* *y Mn*	dies +
	3	dies *y*	*Mn* *Mn*
(4)	1	*Mn* *y Mn*	dies sn^3
$\frac{+\ +\ Mn}{y\ sn^3\ +}$	2	*Mn* *y* sn^3 *Mn*	dies +
	3	dies *y* sn^3	*Mn* *Mn*

* **z**-segregation yields always: $\frac{Mn}{Mn}$

14+ and 2*sn*^{3}, thus making experiment 1 fit in with the others as to the striking prevalence of one type of spot. In order to find out the processes by which the different types of spots were produced, table 11 was constructed; it gives the types of spots to be expected in case of single somatic crossing over in the regions (1) *y*-*sn*^{3}, (2) *sn*^{3}-*Mn* and (3) *Mn*-spindle fibre point and with **(x)** equational or **(y)** reductional segregation of the, daughter fibres. Another possible equational separation, **(z),** will not lead to visibly different spots. It is assumed that no chromatid reduction takes place. The contrary assumption can be disproved as in previous discussions.

An analysis has to be based on a consideration of *all* four experiments. Regarding the + spots in experiment 1 it is seen that three different processes of crossing over and segregation, namely **(1y), (2y),** and **(3x)** lead to + spots. As we expect the same processes in comparable frequencies to occur in experiments 2 to 4, we have to find out from table 11 what kinds of spots would result from these processes.

(a) Process **1y** would lead to *sn*^{3} in experiment 2, + in experiment 3, and *sn*^{3} in experiment 4. Indeed, experiment 2 yielded *sn*^{3} spots as the largest class, in conformity with the + spots constituting the largest class in experiment 1. In experiment 3, however, no + spots were found and in experiment 4 the *sn*^{3} spots constituted the smallest class. Thus process **1y** does not fulfill the requirements.

(b) Process **2y** can be excluded also. It would lead to + spots in all four experiments, while + spots actually form the smallest class in experiment 2 and do not occur at all in experiments 3 and 4.

(c) The remaining process **(3x)** would give results in accordance with all actual findings, as the different kinds of spots to be expected from it represent indeed the most frequent class in each experiment.

A similar analysis, carried out in regard to the less frequent classes of spots leads to the following results: Process **1x,** crossing over between *y* and *sn*^{3} and equational fibre point segregation can account for *y* spots in experiments 1 to 4; process **2x,** crossing over between *sn*^{3} and *Mn* with equational fibre point segregation can account for *y sn*^{3} spots in experiments 1 and 4 and for *y*-*sn*^{3} twin spots in experiments 2 and 3. It is seen in some cases that certain classes of spots are made up from products of different processes, as in the case of *y* spots in experiment 3, where the majority is considered to have originated in consequence of **3x,** while some must be products of **1x.** Furthermore, whenever *y*-*sn*^{3} twin spots are expected, as in experiments 2 and 3, some of these will cover only so small an area as to appear as single *y* or *sn*^{3} spots and will therefore add to the heterogeneity of the *y* or *sn*^{3} classes. In some of these cases the different origin should lead to spots which contain either Minute-n or its normal

allele. As indicated in table 10, both types of spots were frequently found in the different classes and were present in proportions which were roughly in conformity with expectations.

There are a few types of spots which cannot be accounted for on the basis of single crossovers namely in experiment 1, 3 *sn*^{3} spots, 2+ ♂ colored spots; in experiment 2, 4 + spots, 8+ ♂ colored spots; in experiment 4, 2 *sn*^{3} spots.

A discussion of the + ♂ colored spots will be postponed (cf. p. 667.) The remaining few spots can be accounted for by double (and triple) crossing over, as can be readily seen. The occurrence of such multiple cross-

TABLE 12

Number of spots with 1 or 2 and with more than 2 setae in experiments 1–4 from table 10.

EXP.	PREVALENT CLASS		ALL OTHERS	
	1–2 SETAE	>2 SETAE	1–2 SETAE	>2 SETAE
(1)	7	8	11	1
(2)	83	112	45	6
(3)	33	69	13	5
(4)	38	51	7	0
	161	240	76	12
Totals	**401**		**88**	
% of spots >2	60		14	

overs should lead to certain other spots also which would not be distinguishable from the spots produced by the single crossover processes. This adds slightly to the heterogeneity of the main classes.

The following summary of the results of these tests is based on the as yet unproved assumption that apparent non-crossovers are really crossovers between the rightmost locus investigated (*Mn*) and the fibre point. We then find: (1) the most frequent cause of spots is somatic crossing over in the region nearest the fibre point, region 3, and equational segregation of the fibre points; (2) somatic crossing over in other regions of the X chromosomes occurs also and if followed by equational divisions of the fibre points accounts for the less frequent types of spots.

The relative frequencies of the different types of crossovers will be considered after one more result has been pointed out.

If we tabulate (table 12) for experiments 1 to 4 the numbers of spots which cover only 1 or 2 setae and those which cover more than 2 setae, we see that 60 per cent of all spots in the prevalent class and only 14 per cent of all other classes belong to the larger kind. The difference becomes more significant still if we remember that part of the spots in the main class belong in reality to the other group and thus should tend to increase the

number of small spots in the prevalent class. This striking correlation between chromosome region of somatic crossing over and size of spot might be accounted for on two different assumptions (other possible but less probable causes will not be discussed): (a) The products of crossing over to the left of *Mn* might have lower viability than those of crossing over to the right so that their growth is more restricted; (b) the relative frequency of crossing over to the left of *Mn* increases with the age of the developing insect, so that late cell divisions are more frequently preceeded by such kind of crossing over. As the size of the spot area formed decreases the later the time at which segregation takes place, crossovers to the left of *Mn* would result in a relatively high number of small spots.

Assumption (a) can be rejected, as there is no obvious reason why viability differences should differentiate between crossovers to the left or to the right of *Mn*, especially as certain spot constitutions which represent left crossovers in one experiment signify right crossovers in another. This leaves assumption (b) as an explanation of the average size differences of the different types of spots. *In Mn flies the region of somatic crossing over along the X-chromosome is correlated with the developmental stage of the organism.* No such relation was found in the not-*Mn* flies recorded in table 8.

A calculation of the relative frequencies of crossing over in different regions is made very difficult by this finding. It is probable that many cases of segregation after crossing over to the left of *Mn* occur so late that no seta is involved and the mosaic area remains undetected. The relative numbers of observed spots belonging (primarily) to the right and to the left crossover group is therefore biased to the disadvantage of the smaller left crossover spots. How great this bias is can hardly be estimated at present, but it seems very doubtful that it will be great enough to establish a ratio between somatic crossovers to the left and to the right of *Mn* which equals the ratio in germinal crossing over. If equality existed, the chance of observing a left crossover spot would have to be only 1/95 of that of observing a right crossover one, as the germinal ratio is of the order of 19:1 and the observed somatic ratio is of the order of 1:5 (88:401). If this chance is considered too small, it follows that somatic crossing over should be more frequent in the immediate neighborhood of the spindle fibre than in other regions of the X chromosomes. This finding agrees with the similar though less pronounced concentration of crossovers to the right of sn^3 observed in the experiments described in table 9 and table 8. However, such a calculation based on the *total* of observed spots obscures the fact that at different stages of development the relative frequencies of somatic crossovers in different chromosome regions vary.

The correlation between region of crossing over and developmental stage throws light on the divergence of our findings of more than one type of spot and the earlier findings of BRIDGES in which only one kind of spot, explicable by crossing over to the right of *Mn*, had been observed. BRIDGES focussed his attention on the larger spots, not on single seta spots; and he restricted his observation mainly to the head and thorax. As the great majority of spots originating after crossing over to the left of *Mn* comprises only 1 or 2 setae, it is probable that they have been overlooked formerly. Only 12 out of 489 spots summarized in table 12 covered more than 2 setae and only 3 of these occurred on head or thorax. It is not surprising that such a small number of aberrant spots should have gone unobserved.

There is one more point of divergence between the earlier results and interpretations of spots in *Mn* flies and the present ones. It relates to the number of X chromosomes present in cells of the mosaic area. This question will be considered separately.

Experiments involving *y*, *sn*^{3} and "Theta"

No proof has been given as yet for the statement that the apparent noncrossover spots were indeed results of crossing over to the right of the rightmost locus considered. This will be demonstrated now and it will be shown that somatic crossing over is a general process preceeding somatic segregation.

As indicated in the introduction, an experiment was undertaken to determine whether the whole chromosome was lost during the supposed elimination of the *Mn* chromosome. All former findings except those described in the last section had pointed to an "elimination" of at least most of the *Mn* X chromosome—from yellow (0.0) to the right of *Mn* (56.7). There were no "good" loci known to the right of *Mn* which could be tested with respect to their possible elimination. However, MULLER'S finding of an induced, cytologically visible duplication, Theta (θ), had provided a tool for this investigation (PATTERSON 1930). Theta is a deleted X chromosome comprising the left end with the loci yellow, scute, and broad (0.0–0.6) and bobbed from the right end. In the stocks used Theta was attached to the right end of a normal X. The chief property by which the presence of Theta is ascertained is its possession of a normal allele of yellow. In males or females in which the normal X chromosomes contain the recessive mutant yellow, the presence of Theta can be seen by their not-yellow normal body color.

When females of the constitution *y Mn* θ/*y* were inspected for spots of not-Minute setae, the color of these spots was expected to be yellow in case a complete elimination of the *y Mn* θ chromosome had taken place

and to be normal when only the *y Mn* part, but not the *θ* part of the chromosome had been lost. In a first experiment of this type 26 spots were found all not-yellow. This seemed to prove that only a part of the X was eliminated and that a break had occurred between *Mn* and the spindle fibre insertion, where Theta is attached. When, however, females of a similar constitution were examined in which the Theta-fragment was attached to the not-*Mn* chromosome (*y Mn*/*y θ*), it was discovered that the not-*Mn* spots were of two kinds, some being not-yellow, others being yellow. The first kind would be expected from a simple elimination of most or all of the *y Mn* chromosome. But the yellow spots seemed to show that *together with the Mn-containing part of one X chromosome the Theta part of the other X chromosome had disappeared.* A full analysis of these findings will be given in a later chapter. Here we shall follow the implications in regard to mosaics in which no sex-linked Minute is involved.

When somatic segregation had been recognized as the process leading to mosaic formation it was obvious that an interpretation of the Minute-Theta results involved assumptions of somatic crossing over to the right of *Mn*. Accordingly Theta was introduced into females of constitution otherwise similar to that described in the preceding chapter (the presence of *Hw* and dl-49 may be disregarded, but see p. 708). Table 13 contains the results. Two main types of females were inspected: (1) containing *y* and *sn*^{3} in one X and *y* and Theta in the other and (2) containing *y*, *sn*^{3} and Theta together in one X and only *y* in the other one. In these experiments due to the homozygous condition for *y* at the left end of the X chromosomes, crossing over between the X chromosomes, if it occurred at all, could be observed in its effect only when the exchange had taken place between the locus of *sn*^{3} and the attachment point of Theta. Three main possibilities seemed to exist: (a) no somatic crossing over, (b) somatic crossing over occurring at a two strand stage with respect to the two X chromosomes, each chromosome being still represented as a single strand, (c) somatic crossing over at a four strand stage. These might be regarded to be of unequal probability if considered in the light of our foregoing analysis and of cytological results. But the analysis offered a new test of the findings reported above and it was also thought best to judge the genetic evidence of these experiments on its own merit as much as possible. This will be done herewith.

(a) No somatic crossing over. In this case somatic segregation in experiment 1 should have yielded two daughter cells with the constitutions *y sn*^{3} and *y θ* giving rise to *y sn*^{3} spots (*y θ* spots are phenotypically indistinguishable from the surrounding tissue). Only two *y sn*^{3} spots out of 143 spots were found, showing that segregation without crossing over cannot account for the findings.

TABLE 13

Spots in experiments involving primarily y, sn^3 and θ. Part of the individuals contained an autosomal Minute in order to increase the frequency of spots.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	sn^3			y			$y\ sn^3$		y-sn^3		$y\ sn^3$-sn^3	$+\sigma^3$ COL.
				1	2	>2	1	2	>2	1	>2	2	>2	>2	
1a	*y sn³/y θ*	170	53	16	5	20	3	2	—	1	1	—	2	1	2
b	*y sn³ bb/y Hw* dl49 *θ*	268	59	33	10	11	4	—	1	—	—	—	—	—	—
c	*y sn³ bb/y θ*	2558	31*	13	1	7	8	—	—	—	—	1	1	—	—
				62	16	38	15	2	1	1	1	1	3		
Totals Exp. 1		**2996**	**143**	**116**			**18**			**2**		**4**		**1**	**2**
2a	*y sn³ θ/y Hw* dl49	679+	148	87	28	21	6	2	3	—	—	1(?)†	—	—	—
b	*y sn³ θ/y w*	338	39	22	6	1	8	—	1	—	—	—	1	—	—
				109	34	22	14	2	4	—	—	1(?)	1		
Totals Exp. 2		**1017+**	**187**	**165**			**20**			**—**		**2(?)**		**—**	**—**

* In exp. 1c only head and thorax were inspected. All 31 spots were found on the thorax.

† Doubtful if the single bent seta was genetically sn^3.

The same conclusion holds true for experiment 2. Here simple segregation should produce two daughter cells of the constitutions *y sn*^{3} *θ* and *y*, giving rise to *sn*^{3} and *y* twin spots. But only two twin spots (one doubtful!) out of 187 were found.

(b) Somatic crossing over at a two strand stage.

Expectation in experiment 1: two daughter cells with the crossover constitutions *y sn*^{3} and *y*; these should result phenotypically in *sn*^{3} and *y* twin spots.

Result: only 2 such twin spots out of 143 spots.

Expectation in experiment 2: two daughter cells with the crossover constitutions *y sn*^{3} and *y θ* giving rise to *y sn*^{3} spots (the *y θ* area not being distinguishable from the surrounding tissue).

Result: No *y sn*^{3} spot out of 187 spots.

Conclusion: Segregation with crossing over at the two strand stage does not account for the findings.

(c) Somatic crossing over at a four strand stage. This implies for our discussion that only two strands at any one level cross over. Otherwise four strand crossing over would here be indistinguishable from two strand crossing over. Before entering a detailed analysis, it will be shown once more that generally no chromatid reduction occurs in the formation of mosaic areas.

We consider the original constitution of the females in experiment 1 and experiment 2. If reduction occurs in experiment 1 two of the four resulting cells would each obtain a non-crossover strand of the constitution *y sn*^{3}, or *y θ*, and the other two cells would each obtain a crossover strand of the constitution *y sn*^{3} *θ* or *y*. The areas resulting from later divisions of the four reduction products would exhibit the phenotypes *y sn*^{3}, +, *sn*^{3}, and *y*. As the phenotype + is not different from the surrounding not-reduced tissue, the visible result would be a triplet spot with a *y sn*^{3}, *sn*^{3}, and *y* area. No spot of this nature was observed and the *y sn*^{3} phenotype itself appeared not more than three times in 143 spots. As to experiment 2: the four reduction products would have the constitutions *y sn*^{3} *θ*, *y*, *y sn*^{3}, and *y θ*, giving rise to a triplet area with the phenotypes *sn*^{3}, *y*, and *y sn*^{3} as in experiment 1. Again no such spot was found nor a single *y sn*^{3} phenotype out of 187 spots.

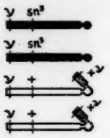
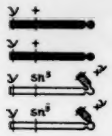
Thus there remains the following mechanism to be examined: crossing over between two of the four strands and subsequent segregation two by two of strands without chromatid reduction. The main consequences of this mechanism are diagrammatically represented in figures 8 and 9. At the left of the first horizontal section (a) the four chromatids are represented which originated as a consequence of crossing over in the *sn*^{3}-Theta-attachment region between two of the original chromatids. To the right of this

type of cross-over (see text)	resulting chromatids	(X)	(Y)	(Z)
(a)		sn³ / +	y / +	+ / +
(b)		y / +	sn³ / +	+ / +
(c)		sn³ / y	ysn³ / +	y / sn³
(d)		+ / +	ysn³ / +	+ / +
(e)		y / +	ysn³ / +	+ / y
(f)		y / +	sn³ / +	+ / +

FIGURE 8. *y sn³/y θ*. (See table 13, experiment 1.) Six types of single crossovers and the results of three different types of segregation.



type of cross-over (see text)	resulting chromatids	(x)	(y)	(z)
(a)		+ / sn³	y / +	+ / +
(b)		y / sn³	+ / +	+ / +
(c)		+ / y	y / sn³	y / +
(d)		+ / +	y / sn³	+ / +
(e)		y / +	y / sn³	+ / y
(f)		y / +	+ / sn³	+ / +

FIGURE 9. *y/y sn³θ*. (See table 13, experiment 2.) Six types of single crossovers and the results of three different types of segregation.

four chromatid group are shown the results of the three different possibilities of distributing the chromatids two by two into two daughter cells. In both experiments the first of these possible types, **x,** leads to the appearance of a *sn*^{3} spot, the second, **y,** to that of a *y* spot while the last, **z,** does not give rise in any of the two resulting cells to a phenotype different from the surrounding tissue. If we compare these deductions with the actual results (table 13) it appears that the great majority of spots seem to fit the expectation. In experiment 1, 134 spots out of 143 and in experiment 2, 185 out of 187 spots exhibit phenotypes which would result from the mechanism discussed. The proportion of *sn*^{3} to *y* spots in both experiments is similar also, namely 116:18 in experiment 1, and 165:20 in experiment 2. Before accepting the conclusion that crossing over to the left of the Theta-attachment and an ensuing chromatid distribution according to two different types in a proportion of about 8:1 is responsible for the findings, it will be necessary to see whether there are other types of crossing over and segregation which would lead to the same observed spots. Five further types of crossing over are listed in figures 8 and 9. In the horizontal sections (b) and (c) the possibility is considered that crossing over involves the right (bobbed) end of the Theta fragment and that part homologous to it which is located near the spindle fibre in the X chromosome—(b) in the free X and (c) in the X chromosome to which Theta is attached. In the sections (d)–(f) the possibility is considered that crossing over involves the left (yellow) end of the Theta fragment and the homologous part in the left end of the X chromosomes— (d)between a Theta chromatid and the chromatid of the X chromosome to which the Theta chromatid is attached, (e) between a Theta chromatid and the sister X chromatid of that to which the Theta chromatid is attached, and (f) between a Theta chromatid and a chromatid of the free X chromosome. If somatic crossing over obeys the same rule as does germinal crossing over, then it can be predicted that the crossover processes represented in sections (b)–(f) will play only a very small role, if any, in comparison to the crossover type referred to in (a). This follows from the generally established fact that short duplications take very little part in germinal crossing over (DOBZHANSKY 1934). In addition there may be quoted the results of unpublished experiments on germinal crossing over in the X chromosomes of females possessing one Theta attachment: Only 2 out of 7559 individuals occurred as a result of crossing over between Theta and the homologous region near the spindle fibre of an X chromosome. But in spite of the rareness of such crossing over types during germ cell formation, we have to consider the consequences of their possible occurrence in somatic tissues if followed by segregation. For, lacking any previous acquaintance with

somatic crossing over, we are not justified at this stage to apply our knowledge of germinal processes to somatic ones.

The procedure will be to tabulate which types of crossing over and segregation are able to give rise to the two main kinds of spots which were observed, namely *sn*^{3} and *y*.

It is seen (fig. 8) that *sn*^{3} spots originate in experiment 1 only according to the types **ax, by,** and **fy.** In experiment 2 (fig. 9) *sn*^{3} spots can originate according to types **ax** and **fy.** Other types in which *sn*^{3} appears as part of a twin segregate will not be considered for the moment.

The process **by** in experiment 2 does not give rise to *sn*^{3} spots. It therefore seems sufficient to consider only **ax** and **fy** as possibly responsible for the observed *sn*^{3} spots. Now the process **fy** should hardly occur without the occurrence of **dy** and **ey,** for a striking preference of the Theta fragment for crossing over with different kinds of the distant yellow ends of X chromosomes is not likely. However, **dy** and **ey** give rise to *y sn*^{3} spots in experiment 1 and to *y* and *sn*^{3} twin spots in experiment 2. Such spots were negligible in number—and they can also originate through different processes. It is therefore concluded that no appreciable percentage of **fy** processes occurred and that the majority of *the sn*^{3} *spots both in experiment 1 and 2 were produced as results of the process* **ax.**

Yellow spots would appear as a result of the following processes in experiment 1: **ay, bx, ex, ez,** and **fx.** In experiment 2 yellow spots will result from **ay, cx, cz, ex, ez,** and **fx.** The processes **ey, ex, ez,** and **fx** agree in both experiments in yielding only *y* spots. On the other hand, **bx** would be responsible for *y* and *sn*^{3} twin spots in experiment 2, and **cx** for *y* and *sn*^{3} twin spots in experiment 1. But twin spots have to be considered also, as some of them will appear phenotypically only as *y* areas (others as *sn*^{3} areas). Besides **bx, cx,** and **cz** we have, therefore, to discuss still the processes **cy, by,** and **ey,** which give rise to *y* and *sn*^{3} twin spots in experiment 2. The last named group of processes, if they occur, gives rise to *y sn*^{3} spots in experiment 1. Actually only 2 *y sn*^{3} spots out of 143 were found in experiment 1 so that these processes at best can account only for a small number of *y* spots in experiment 2 and cannot account at all for any of the 18 *y* spots in experiment 1. We are then restricted to an analysis of the types **ay, bx, cx, cz, ex, ez,** and **fx.** Do all these crossing over and segregation processes occur? A decision cannot be reached without taking recourse to other data. We have seen in the preceding chapter on spots in *Mn* or Bld-bearing females that the occurrence of types of segregation which are reductional with respect to sister fibre attachment points could be excluded. If we use this knowledge for our present problem we see that the process **ay** representing such a reductional segregation becomes eliminated

and only equational segregation processes remain. They all have in common the fact that they involve crossing over between the Theta duplication and the X chromosome, **bx, cx** and **cz** crossing over in the homologous right regions and **ex, ez** and **fx** in the homologous left regions. Whether both kinds of crossovers contribute to the *y* spots or if only one kind occurs and if so, which one it is, cannot be ascertained from the present experiments. But further information will be yielded in an analysis of experiments which involve *y*, *sn*^{3}, *Mn*, and θ. No discussion will be devoted to the *y sn*^{3} spots nor to the *sn*^{3} and *y sn*^{3} twin spot. Simple elimination, non-disjunction processes, or multiple crossovers at one or successive stages may be involved.

The results of this chapter can be summarized as follows:

(1) Somatic segregation is always (or nearly always?) preceded by somatic crossing over between two of four chromatids.

(2) If the result of a preceding section is held to be generally true, namely, that segregation is always equational with respect to spindle fibre attachments, then it is found that somatic crossing over involves not infrequently the Theta-duplication which only very rarely participates in germinal crossing over.

On the basis of these findings it is postulated that somatic crossing over underlies segregation also in those cases where the genetic constitution, on account of the absence of an attached Theta duplication, does not seem to allow a direct test of crossing over in the rightmost region. Such a test, however, is possible under some circumstances as will be shown in the following section.

Experiments involving bobbed as a means of determining the rightmost crossover region

As pointed out before, the fact had been unexpected that in flies heterozygous for bobbed (*bb*) or one of its alleles "elimination" of the not-bobbed X chromosome generally does not result in spots exhibiting the bobbed phenotype. Table 14, experiments 2 and 3, contains data on such spots in flies which had *Mn* in one X and *bb* or *bb*^{l} (a lethal allele of *bb*) in the other one. It is seen that 34 of the spots in experiment 2 possessed phenotypically non-*bb* setae, and that in experiment 3 where only non-*bb* spots would have been visible, 10 spots were found. Leaving aside at present the spots with smaller than normal setae, the non-appearance of the *bb* character in these spots has to be explained.

The assumption of non-autonomous development of the bobbed character from cells of a mosaic area, although possible, seemed unlikely, especially in view of the result of an extensive test of the sex-linked factor "tiny bristle" (*ty*, 1, 44.5) which is very similar in action to bobbed, to-

TABLE 14

Seta length in spots of individuals containing bb and bb^1 (exp. 2 and 3). Experiment 1 is the control.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	TYPE OF SPOT (LENGTH OF SETAE) +	TYPE OF SPOT (LENGTH OF SETAE) <+
1	$\frac{+\ g^2\ Mn}{y\ +\ +}$	528	58	54	4†
2	$\frac{+\ g^2\ Mn\ +}{y\ +\ +\ bb}$	769	45	34	11*
3	$\frac{+\ g^2\ Mn\ +}{w^e\ +\ +\ bb^1}$	476	10	10	?

† Only spots are recorded which enclose macrochaetae on head or thorax. They were *y* in exp. 1 and 2 and $+^M$ in exp. 3. In the latter experiment spots with setae shorter than $+^M$ could not be distinguished from the rest of the body.

‡ Setae length as in *bb* ♀ : 2 spots.
Setae length > "*bb* ♀," but <+: 2 spots.

* Setae length as in *bb* ♀ : 4 spots.
Setae length > "*bb* ♀" but <+: 6 spots.
Setae length like $bbXO♂$: 1.

gether with which it can be classified as a recessive Minute. The test consisted in observing whether *ty* behaved autonomously in spots or not. The result was that it did, which made the discordant behavior of *bb* more surprising.

The theory of somatic crossing over in the form proposed allows us to understand the apparently exceptional behavior of *bb* if we make a specifying assumption concerning the region of somatic crossing over in the X. It has been pointed out before that in the majority of cases this region lies between the locus of *Mn* and the right end of the X. If the point of crossing over were located between the rightmost factor, which is bobbed, and the right end of the X, then the behavior of the phenotype bobbed in spots would be exceptional also under the somatic crossing over theory, for the equational segregation of fibre attachment points is by necessity reductional for all loci to the left of the crossover point. Therefore one of the segregation products of a fly which is heterozygous for bobbed becomes homozygous for this locus. Thus in a *Mn*/*y bb* female crossing over at the four strand stage, if occurring to the right of *bb*, would lead to daughter cells of the constitutions *Mn*/*Mn* and *y bb*/*y bb*, the latter giving rise to *y* spots. These spots, in spite of their assumed homozygous condition for *bb*, show long not-*bb* bristles. Or let us consider experiment 4 of table 5. In females of the constitution *y* . . . bb^1/sn^3bb^z, four strand crossing over

to the right of the *bb* alleles should yield daughter cells of the constitutions *y . . . bb^{l}/y . . . bb^{l}* and *sn^{3}bb^{z}/sn^{3}bb^{z}*. From the knowledge of the recessive lethal action of *bb^{l}* in zygotes the most likely assumption would be a cell lethal action which hinders the *y . . . bb^{l}/y . . . bb^{l}* cell from developing into a spot area. However, two *y* areas in *y-sn^{3}* twins were found covering 3 setae and four covering 2 setae besides numerous one-seta *y* areas (tables 7 and 5). The difficulties disappear if we assume that the point of somatic crossing over lies to the left of the *bb* locus. This assumption agrees with the fact that as a rule no *germinal* crossing over occurs between *bb* and the fibre locus (STERN 1929). Crossing over to the left of *bb* will leave the constitution of the daughter cells unchanged with regard to *bb*. In *Mn/y bb* flies the daughter cells will be *Mn +/Mn bb* and *y +/y bb* and the resulting *y* spot, heterozygous for *bb* like the rest of the fly, will have not-*bb* bristles; in *y . . . bb^{l}/sn^{3}bb^{z}* females the twin spots will have the constitutions *y . . . bb^{l}/y . . . bb^{z}* and *sn^{3}bb^{l}/sn^{3}bb^{z}*, again not differing from the surrounding tissue in regard to *bb*.

There remains to be given an explanation of the 11 spots in experiment 2 which exhibited *y* setae of shorter than normal length. That they are not due to a special behavior in regard to *bb* is probable from the control experiment 1 in which *bb* was not involved at all and in which 4 spots with shorter setae were found. These 4 spots are to be expected as results of crossovers between *y* and *Mn* thus producing spots of the constitution *y Mn/y +*, the variation in setae length representing the known variability of *Mn* setae in the presence of different modifiers (which in these spots must be of varying combinations due to different crossovers). The same explanation is adopted for 10 of the 11 spots with smaller than normal setae in experiment 2, while the single bristle spot with a very short, *bb* XO-like seta was perhaps due to a real elimination of the *Mn* X chromosome. The higher frequency of the smaller spots in 2 than in 1 is within the limits of variability of somatic crossing over (the difference also being smaller than three times the standard error). It might be added that experiments 2 and 3 were conducted in such a way as to exclude the presence of a supernumerary Y chromosome which would have covered the *bb* effect.

The assumption that the crossover point lies always to the left of *bb*, can be applied to an independent test of the origin of the *y* spots which appear in the Theta experiments discussed in the preceding section. We had seen that the *y* spots may have resulted both from a reductional segregation process after crossing over between the X chromosomes (**ay** process) and from equational segregation processes after crossing over involving the Theta duplication (processes **bx . . . fx, cz, ez**), The first alternative could

not be rejected on the basis of an analysis of the pertinent data but only on the basis of evidence from earlier experiments.

If the *y* spots occurred in consequence of processes **ay,** after reductional distribution of the fibre points, then a reductional distribution for the *bb* locus would have occurred also. Bobbed then should exhibit its phenotype in suitable spots. But if the *y* spots were due to any of the processes **bx . . . fx, cz, ez,** then *bb* should not appear phenotypically. This point could have been tested in experiments 1b and 1c of table 13. In experiment 1b five *y* spots occurred. A reliable distinction of *bb* and not-*bb* setae generally can be made only in macrochaeta of the head and thorax and in flies which do not carry a Minute factor. Only 3 of the 5 *y* spots occurred in non-Minute flies and none included head or thorax macrochaetae. Experiment 1c represents a special attempt to obtain *y* spots covering suitable setae. Of the nearly 3000 individuals inspected only 18 possessed *y* head-thorax spots, 4 of which covered macrochaetae. Three of these spots had setae which were clearly of about normal, not bobbed size; one had setae of a size similar to *bb*. This last named *y* spot seems to indicate the occurrence of the **ay** process. However, too much weight should not be laid on this single finding which is contradicted by sufficient other evidence. It could, for instance, be regarded as a case of crossing over to the right of *bb* with normal disjunction of the fibre points. However, this is not suggested as a probable explanation.

The other three not-bobbed, yellow spots establish definitely the occurrence of at least some of the crossing over and segregation processes **bx . . . fx, cz, ez.** It is probable that one or both of the processes **b** and **c** were involved, as the bristle size in these spots, though not-bobbed, was apparently somewhat abnormal. This points to a 3X (superfemale) constitution of the spots, as to be expected from **bx** and **cx** or **cz** (fig. 8).

The number of X chromosomes in cells of spots

If mosaic spots in females which do not contain a duplication originate by segregation of four chromatids two by two following crossing over between two X chromosome chromatids, then the X chromosomes in cells constituting the mosaic regions must be present in the diploid number This expectation is in contrast to that derived from a simple elimination hypothesis which leads to the expectation of finding one X chromosome only. Still other numbers of chromosomes can be predicted after segregation in females carrying a duplication as can be seen in figures 8 and 9. An investigation of the number of X chromosomes in mosaic spots should provide new tests for the proposed theories.

It was not thought possible to determine the number of X chromosomes

by a cytological study as no cell divisions are expected in the hypodermal cells which constitute the spots of adult individuals. Genetic means, however, are available. BRIDGES (1925) used two criteria: the presence (♂ = X) or absence (♀ = XX) of a sex comb in case of a spot covering the sex comb region of a front leg and the shade of w^e (eosin 1, 1.5) color in eye spots of individuals which according to their genetic constitution exhibited eosin in a mosaic eye part. Eosin is known to be pink in females and pinkish yellow in males. Since the sex comb region is involved only very rarely in a spot and since the eosin test allows for reliable judgments of the eye color only in comparatively large spots, a third criterion for X or XX condition was used primarily in the present studies, namely, the coloration of the fifth and sixth abdominal segment.[1] These segments are dark in males and, in most stocks, colored only along the posterior margins and near the median line in females. This coloration is cell-autonomous as many cases ranging from smallest to largest black spots on female segments in certain experiments show. A fourth criterion of the X or XX constitution of spots can be derived from a consideration of bobbed spots as will be shown in the next section.

The determination of the sex of spots as an index of the number of X chromosomes was made in all later experiments after the importance of this condition was recognized. In the earlier experiments the determination of sex was not made while the flies were alive. However, it seems certain to me that I would have recorded the fact of male coloration in abdominal spots had it occurred, as I wrote down individual records for each spot noting the numbers of bristles involved, the abdominal segment, and often added a sketch of the spot. Whenever no comment was made in my notes (in 22 out of 147 cases) it can be regarded as highly indicative of the absence of male coloration, that is, of the presence of two X chromosomes.

Consequently all those spots found in the experiments discussed in the preceding sections of this paper in which two or more setae of the critical abdominal region were involved have been tabulated in table 15. Besides, the results of three further experiments reported elsewhere (STERN 1935b) have been added. It will be noted that the mosaics have not been classified under the alternative "female color or male color" but rather under "male color or no male color." The latter is better, since it is possible that in rare cases very narrow spots do not exhibit male coloration in spite of 1X constitution even if they cover two or more setae. The table shows that the great majority of mosaics, 132 out of 147, did not exhibit male coloration. They thus contain cells with the constitution XX, in agreement with the

[1] Owing to the fact that the apparent first segment is regarded to be originally composed of two segments, the morphologically fifth and sixth segments are the ones which appear as fourth and fifth without knowledge of the homologies.

theory of crossing over and segregation and in contradiction to the elimination theory. Fifteen spots exhibited male coloration, an effect of one X constitution.

The probable origin of most of these exceptional spots will be discussed later in connection with the influence of an extra Y chromosome on somatic crossing over. It is significant that no male color appeared in any of the 31 spots which exhibited the typical result of segregation, the twin condition. Here it is pointed out only that the male-colored spots occurred in a few very nearly related cultures in each experiment: 5 of the 6 male spots in line 1 of table 15 came from 3 related cultures of a total of 34, all 3 male

TABLE 15

The sexual coloration in critical abdominal spots. Numbers in ( ) represent those of the total number of spots which were recorded without a comment as to sexual coloration.

EXPERIMENTS	MAIN FACTORS INVOLVED	*sn*³		*y*		*y sn*³		*y-sn*³		+	NO ♂ COL. : ♂ COL.
		NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	
Table 5, 2–4†	*y*, *sn*³	7(2)	3	7(3)	3	—	—	22(1)	—	—	36:6
Table 8, 1–4	*y*, *sn*³	—	—	9	—	13	1	—	—	2	22:3
Table 10, 1–4‡	*y*, *sn*³, *Mn*	—	—	2	—	7	—	—	—	2	9:2
Table 13, 1a, b	*y*, *sn*³, *θ*	12(3)	—	1(1)	—	—	1	—	—	2	13:3
Table 13, 2a*	*y*, *sn*³, *θ*	9(9)	—	4(3)	—	—	—	—	—	—	13:0
y Hw/*sn*³		1	1(?)	—	—	—	—	9	—	—	10:1(?)
y Hw dl-49/+		—	—	16	—	—	—	—	—	—	16:0
(*y sc*)⁻/*sn*³		13	—	—	—	—	—	—	—	—	13:0
									Total:		132(22):15(?)

† No abdominal spots were recorded in exp. 1.

‡ No critical spots occurred in exp. 3; for exp. 2 see text.

* No critical spots occurred in exp. 2b.

spots in line 2 from one single culture of a total of 29 and both male spots in line 3 from one single culture of a total of 32. This indicates that special genetic conditions were responsible.

The two normal, male-colored spots in line 4 will be commented upon later.

A comprehensive experiment involving y, sn³, Mn and Theta

Each of the experiments dealt with in the preceding chapters yielded results which, taken together, furnish a consistent picture. However, some points were cleared up only by referring from one experiment to another and other points were left completely unsolved. The present chapter gives an account of experiments in which *y*, *sn*³, *Mn*, and *θ* were involved at the same time. Emphasis will be laid on two questions: (1) Are the *y* spots

TABLE 16a

Spots in experiments involving primarily y, sn^3, Mn, and θ.

EXP.	CONSTITUTION	IND.	SPOTS	FREQUENCY IN % OF ALL SPOTS	% y SPOTS	y 1	y 2	y >2	sn^3 1	sn^3 2	sn^3 >2	+ 1	+ >2	+ ♂ COL.	y sn^3 1	y sn^3 >2	y-sn^3 2	y-sn^3 >2	OTHER SPOTS
1a	$\frac{y + + Mn +^{bb}}{y\ w^e\ sn^3 + \theta}$	219	82	38	7	2	1	12	19	3	42	—	1	—	—	—	—	2	—
b	$\frac{y + + Mn\ bb}{y\ w^e\ sn^3 + \theta}$	1384	285	21	5	30	5	32	93	26	91	—	—	—	—	—	1	5	2*
c	$\frac{y + + Mn\ bb'}{y\ w^e\ sn^3 + \theta}$	221+	59	27	10	8	1	14	15	8	10	1	—	—	—	1	—	1	—
	Totals Exp. 1				**5.8**		**105**			**307**			**2**	—		**1**		**9**	**2**
2a	$\frac{y\ w^e\ sn^3\ Mn +^{bb}}{y + + + \theta}$	97	6	6	5	3	—	2	—	—	—	—	—	1	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ bb}{y + + + \theta}$	260	13	5	3	2	1	6	1	—	—	1	2	—	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ bb'}{y + + + \theta}$	139	19	14	10	8	2	4	1	1	1	2	—	—	—	—	—	—	—
	Totals Exp. 2				**5.6**		**28**			**4**			**5**	1	—	—	—	—	—
3a	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + +^{bb}}$	857+	163	19	4	22	3	5	64	25	40	—	—	—	1	1	—	2	—
b	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + bb}$	95	16	17	2	2	—	—	3	3	8	—	—	—	—	—	—	—	—
	Totals Exp. 3				**3.4**		**32**			**143**		—	—	—		**2**		**2**	—
4a	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + +^{bb}}$	557	41	7	5	16	7	4	5	2	4	1	—	2	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb}$	348	29	8	4	12	—	3	2	3	3	—	1	5	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb'}$	221	19	9	5	4	4	2	4	1	2	—	—	2	—	—	—	—	—
	Totals Exp. 4				**4.6**		**52**			**56**			**2**	**9**	—	—	—	—	—
	Totals Exp. 1–4	**4398+**	**732**																

* 1 spot showed thick heavy bristles of *M* length, the other was a sn^3 (very small seta) and sn^3 twin spot.

which occur in experiments involving Theta really results of crossing over between Theta and the X chromosome instead of results of crossing over between the X chromosomes and subsequent reductional separation of sister spindle points? (2) Do both the left and the right region of the Theta duplication cross over with their homologous regions in the X chromosome?

Four different combinations of *y*, *sn*^{3}, *Mn*, and Theta were investigated (table 16a). In all experiments eosin was involved also and in each of the four combinations different sub-groups were present with either the normal allele of bobbed, or two mutant alleles similar to each other, *bb* or *bb'*. (*bb'* has not as yet been described). No consistent differences between the

TABLE 16b

Spots (from table 16a) which could be classified according to seta length.

EXP.	$y+^M$		$y\ M$		sn^3+^M		$sn^3\ M$		y-sn^3
	1	>1	1	>1	1	>1	1	>1	>
1	10	20	3	1	30	92	10	—	3*
2	—	6	—	1	1	2	—	—	—
3	1	1	—	—	8	22	8	—	2†
4	1	2	1	—	—	—	1	—	—

* $y+^M$–sn^3+^M: 1 spot.
$y+^M$–length of sn^3 not determinable: 1 spot.
$y\ M$(?)–length of sn^3 not determinable: 1 spot.
† sn^3+^M–length of *y* not determinable: 2 spots.

sub-groups are apparent. The frequencies of total number of spots per hundred individuals varied between 5 and 38, but a consistent grouping is clear, giving the highest frequencies to experiment 1, the next to experiment 3, while experiments 2 and 4 present the lowest figures. Striking differences between the relative frequencies of the different types of spots, especially the *sn*^{3} and *y* spots are also obvious. While in table 16a all *y* spots and all *sn*^{3} spots were listed together regardless of their length, table 16b shows that among both groups there were spots with bristles of about normal length as well as of Minute length. An accurate determination of length in spots occurring on the abdomen or including only microchaetae was not possible in general, so that the majority of spots could only be classified in regard to *y* or *sn*^{3}. However, table 16b indicates that the great majority of spots were not Minute. Furthermore all 19 *sn*^{3} Minute spots were single bristle spots so that it seems probable that all or most of the larger *sn*^{3} spots were not Minute, and even among the single bristle spots the majority of the head-thorax spots were not Minute. This holds primarily for experiments 1 and 2. The bristles classified as not-Minute were

TABLE 17

Spots resulting from **x** *(equational) and* **y** *(reductional) segregation in experiments involving y, sn³, Mn and θ.*

CROSSOVER REGION	EXP. 1 $\frac{y + Mn}{y\ sn^3 + \theta}$ (x)	(y)	EXP. 2 $\frac{y\ sn^3\ Mn}{y + + \theta}$ (x)	(y)	EXP. 3 $\frac{y + Mn\ \theta}{y\ sn^3 +}$ (x)	(y)	EXP. 4 $\frac{y\ sn^3\ Mn\ \theta}{y + +}$ (x)	(y)
(a) *sn³-Mn*	*sn³ Mn*	+	*sn³ Mn*	+	*sn³ Mn*	*y*	*sn³ Mn*	*y*
(b) *Mn-θ*	*sn³*	*y Mn*	+	*y Mn*	*sn³*	*y Mn*	+	*y Mn*
(c) right end of θ and of X chromosome bearing θ	*y*(3X) dies		*y*(3X) dies		*y*(*M*/*M*/+) *sn³*/$\underline{\theta\theta}$		*y*(*M*/*M*/+) *sn³*/$\underline{\theta\theta}$	
(d) right end of θ and of free X chromosome	*y*(*M*/*M*/+) *sn³*/$\underline{\theta\theta}$	dies +(3X)	*y*(*M*/*M*/+) +/$\underline{\theta\theta}$	dies +(3X)	*y*(3X) dies	*y*(*M*/*M*/+) *sn³*/$\underline{\theta\theta}$	*y*(3X) dies	*y*(*M*/*M*/+) +/$\underline{\theta\theta}$
(e) left end of θ and of X chromosome (3 possibilities)	*y Mn*		*y Mn*		*y Mn*		*y Mn*	
(f)	—		—		—		—	
(g)	*y Mn*		*y Mn*		*y Mn*		*y Mn*	

often found to be somewhat abnormal, slightly thickened or shortened in varying fashion. The causes for this will soon become clear.

If one tabulates for all four experiments the expectations for spots derived after different types of crossing over and segregation, a relatively simple explanation of the results appears. An abbreviated list of expectations is presented as table 17. Only single crossovers are considered and only the **x** and **y** type of segregation, the first being equational for the fibre points, the second reductional. The **z** type which is also equational does not lead to visible spots after single crossing over between the X chromosomes.

Process **a.** As the table indicates, crossing over between *sn*^{3} and *Mn* with normal disjunction of the fibre points **ax** leads to *sn*^{3} *Mn* spots in all four experiments. Non-disjunction of the fibre points **ay** leads to normal bristle spots in experiments 1 and 2 and to *y* not-*Mn* spots in experiments 3 and 4. No other types of crossing over and segregation represented in table 17 besides **ax** result in *sn*^{3} *Mn* spots. These spots, in all four experiments, are therefore regarded as produced by the process **ax** (the actual non-occurrence in experiment 2 is probably due to the comparatively small number of flies inspected). In good agreement with the findings in the earlier experiments involving *y*, *sn*^{3}, and *Mn* is the fact that somatic crossing over to the left of *Mn* yields only single seta spots. This is interpreted as before to mean that crossing over away from the fibre region occurs only late in development.

The occurrence of the process **ay** can at most account for the + spots in experiments 1 and 2 and for the *y* spots in experiments 3 and 4. However it is more probable that these spots owe their existence to different processes to be discussed below.

Process **b.** Crossing over between *Mn* and the end of the X and equational segregation for the fibre points **(bx)** leads to *sn*^{3} not-*Mn* spots in experiments 1 and 3, to normal bristle spots in experiments 2 and 4.

Spots which are *sn*^{3}+ may be expected also from some of the processes **c** and **d.** It is seen that segregation products in **c** and **d** will contain one X chromosome and two Theta duplications. Whole individuals of this constitution are not viable (unpublished results). In case hypodermal areas of this constitution are able to survive one would expect to find male sex indications in suitable spots. Actually, as will be shown soon, the great majority of *sn*^{3}+^{M} spots did not exhibit male characteristics. This leads us to the conclusion that most of the *sn*^{3}+^{M} spots in experiments 1 and 3 owe their origin to the process **bx.** One would expect a high corresponding number of normal bristle spots in experiments 2 and 4. Only few such spots were found although their relative frequencies in experiments 2 and 4 are higher than in experiments 1 and 3. However, on account of the great

variability in the occurrence of larger sized spots in the head-thorax region in different experiments no serious obstacle is seen in this lack of normal spots which are only visible under the conditions just stated. And it is significant in this respect that of the 100 *y* or sn^3 spots in experiments 2 and 4 only 15 could be classified in regard to seta length. While this type of crossing over to the right of *Mn* with normal segregation can account for the majority of sn^3 not-*Mn* spots, non disjunctional segregation of the fibre points **by** can at most account only for the small number of *y Mn* spots and other processes will also yield this type of spot.

Processes **c, d.** An explanation for the great number of $y+^M$ spots and their varying percentage in the different experiments is provided by the assumption of crossing over between the right end of an X chromosome and the homologous part of the Theta duplication. Two main types of crossovers are possible and assumed to occur with equal frequencies: process **c,** crossing over between Theta and an X chromatid with the Theta duplication; and process **d,** crossing over between Theta and a free X chromatid.

It is seen that normal disjunction **(x)** of the fibre points after crossing over **c** in experiments 1 and 2 yields triple-X *y* spots which possess *Mn* in one of the three X chromosomes. Such a constitution *M*/+/+ is known to produce not-Minute bristles, while the twin segregate whose single X chromosome contains *Mn* is inviable. In experiments 3 and 4 crossing over **c** and normal segregation leads to sister cells, one with 3 X chromosomes and one with one X chromosome and two Theta duplications. The cell with three X chromosomes carries two *Mn* factors. As individuals of such constitution as well as individuals with one X and two duplications are not viable it is assumed that hypodermal segregation products of these constitutions will at best be of low viability and only rarely give rise to spots. No reductional segregation for the spindle fibre points is expected to occur after crossing over of type **c,** as this would mean non-disjunction of the two complete non-crossover sister chromatids which do not carry Theta, a process which throughout this paper has been shown either not to occur at all or at best as an extremely rare exception.

Crossing over **d** with equational segregation **(x)** in experiments 1 and 2 leads to the similar types of inviable or barely viable twin cells with triplo-X *Mn*/*Mn*/+ and X+^{M} cells with two Theta duplications, but in experiments 3 and 4 to triplo-X yellow *Mn*/+/+ cells and inviable X *Mn* partners.

Reductional segregation **(y)** leads to triplo-X cells with normal appearing bristles in experiments 1 and 2 and to combinations with low viability in experiments 3 and 4. Spots with these phenotypes are of course to be expected also from other processes.

Altogether equational segregation after crossing over **c** leads to *y* spots in experiments 1 and 2 and after crossing over **d** to *y* spots in experiments 3 and 4. The triplo-X *Mn*/+/+ constitution of these spots agrees with the somewhat abnormal appearance of the *y*-bristles, the triplo-X condition making for heavier than normal bristles, the one *Mn* possibly accounting for the slight decrease in bristle length.

The frequencies of *y* spots in experiments 1 and 2 should be higher than in experiments 3 and 4 depending on the degree of viability of triplo-X *Mn*/*Mn*/+ cells. If this viability is zero, the percentage of *y* spots in experiments 1 and 2 would be twice that in experiments 3 and 4, for one of the two normal disjunctional types of segregation, **(z),** does not lead to visible spots in experiments 3 and 4 while it is identical in effect with **x**-segregation in experiments 1 and 2. The actual average frequencies are 5.7 per cent for experiments 1 and 2 and 4.0 per cent for experiments 2 and 3. (These figures are based on total *y* spots and are not corrected for the presence of *y Mn* spots.) Calculation shows that such a difference would follow if the chance of finding a *y Mn*/*y Mn*/*y* spot is about one-third of that of finding a *y Mn*/*y*/*y* spot. While this result points to a definite viability of triplo-X spots with two *Mn* factors, not too much weight should be attached to the value obtained.

Processes **e–g.** Finally we have to consider the possibility of crossing over between the left end of Theta and the homologous left end of the X chromosomes. Assuming only equational fibre segregation, two of these processes, **e** and **g,** can lead to the production of *y Mn* spots, while process **f** will not yield visible spots. This represents an alternative to the reductional process which also can account for *y Mn* spots. As the occurrence of reductional somatic segregation has been excluded or made improbable in other cases, it is assumed that the *y Mn* spots are products of equational segregation following the processes **e** and **g.**

Summarizing, we can state that equational segregation of fibre points after single crossing over between the X chromosomes in the region from *Mn* to fibre point and crossing over between the right end of an X chromatid and Theta with equational segregation can account for the great majority of spots, namely the sn^3+^M and $y+^M$ spots. Crossing over with equational segregation occurs also to the left of *Mn*, at a low frequency, as witnessed by the sn^3Mn spots which are products of crossing over in the sn^3 to *Mn* region; proof of crossing over in the w^e to sn^3 region is seen in the occurrence of one out of four sn^3+^M spots which all enclosed eye or ocellar areas but of which the one did not show eosin eye color.

Crossing over between the homologous left regions of the X chromosome and the Theta duplication with equational fibre segregation is assumed to occur and to account for the *y Mn* spots. No detailed attempt will be made

to explain the origin of the remaining classes of spots, which all have few representatives only. Multiple crossing over and occasional new crossover processes in descendants of crossover cells will account for them as it will also for a fraction of the main classes.

The high frequency of crossing over between Theta and the homologous right end of the X chromosomes is surprising in view of the rareness of this process in germinal crossing over. In order to get an estimate, however inaccurate, of this frequency the data of experiment 1 may be considered. The frequencies of *y* spots and of sn^3 spots are 105 and 307. A certain percentage of the *y* and of the sn^3 spots is *Mn*. If the figures of table 16b are taken as a basis for estimating the $y+^M$ and $sn+^M$ spots—a procedure which is doubtful if viewed in the light of our findings concerning the different ontogenetic time at which different crossover processes occur, we find $(30 \div 34)105 = 93$ *y*+ spots and $(122 \div 132)307 = 284$ sn^3+^M spots or about 1 *y*+ : 3 sn^3+. This means that out of each four crossovers in the right end regions one involves the Theta duplication. This is considerably higher than the relative frequency of Theta crossovers in similar experiments without the presence of *Mn* (table 13). However it is still far from what would result from chance crossing over within a multivalent between the four right ends of the X chromosomes and the two Theta duplications. Excluding sister strand crossing over there would be the following types of crossing over: 4 cases of "X with X"; 4 cases of "free X with Theta"; 2 of "duplication-X with the one Theta duplication"; 2 of "duplication-X with the sister Theta duplication." Assuming normal disjunction of fibre points in 50 per cent of the first group, sn^3+^M spots would result. No visible spots would occur in consequence of crossovers according to the second and third group, but in all cases of the last group $y+^M$ spots would be produced. Chance pairing thus would lead to a 1 : 1 proportion of $y : sn^3$ spots instead of the observed 1 : 3 relation.

It is probably significant that crossing over involving Theta is rare in germinal cells where there is a high frequency of crossovers away from the attachment region, while such crossing over occurs frequently in somatic crossovers of flies without *Mn* (table 13) where crossing over becomes concentrated in the proximal region. It is significant that Theta crossing over is relatively still more frequent in the present experiments where, under the influence of *Mn*, the shifting of crossing over to the attachment region has been increased.

As judged by the rareness of *y Mn* spots the frequency of crossing over involving the left end of the Theta duplication is considerably lower than that involving the right end of the duplication.

In table 18 the information as to the sex of spots in this series of experiments is presented. At least 21 out of 25 sn^3 spots were female, as was to

be expected after crossing over between the X chromosomes. All three critical *y* spots were probably female in constitution again as expected, as was one w^e-colored eye-spot which did not affect any setae. Besides these female spots, however, 4 sn^3 male spots and 10 male-colored spots with normal setae were found. It seems probable that these spots represent occasional survivals and division products of 1X cells with two Theta duplications produced from crossing over between the right end of an X chromosome and Theta. The same explanation applies to at least two of the three male-colored spots recorded in line 4 of table 15.

TABLE 18

The sexual coloration in critical spots from experiments involving y, sn^3 Mn, and θ (see tables 13, 17).

EXP.	sn^3 (ABDOMINAL)		w^e sn^3		+	w^e	y	
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♀ COL.	NO ♂ COL.	♂ COL.
1a	3	—	—	1	—	—	—	—
b	15	2	—	1	—	—	—	—
c	1	—	—	—	—	—	—	—
2a	—	—	—	—	1	—	—	—
3a	2	—	—	—	—	1*	—	—
4a	—	—	—	—	2	—	—	—
b	—	—	—	—	5	—	1(?)	—
c	—	—	—	—	2	—	2(1?)	—
Total	**21 ♀**	**2♂**	—	**2**	**10**	**1**	**3**	—

* No setae affected.

It is in agreement with this assumption that the sn^3 male spots are not found in experiments 2 and 4 where they are not expected and that likewise the + male spots are not found in experiments 1 and 3 where they should not occur. The very small numbers of critical spots in experiments 2 and 3 diminish somewhat the weight of these facts.

The analysis of this group of experiments furnishes independent evidence of:

(1) the occurrence of equational segregation of the fibre points as the main, if not the only type;

(2) the frequent occurrence of somatic crossing over between the homologous right regions of the Theta duplication and the X chromosomes;

(3) the lower frequency of somatic crossing over between the homologous left regions of the Theta duplication and the X chromosomes and

(4) the low frequency of crossing over away from the fibre point region in experiments involving *Mn*.

Points (2) and (4) are probably connected causally.

AUTOSOMAL MOSAICS

Somatic autosomal crossing over and segregation

As has been referred to earlier, spots affecting autosomal characters have been found to occur in flies which carry autosomal Minutes. In a pre-

TABLE 19

Frequencies of autosomal spots in experiments with My, Mw, Mβ, and M33j.

CONSTITUTION	M-INDIVIDUALS ♀	M-INDIVIDUALS ♂	SPOTS ♀	SPOTS ♂	SPOTS SEX?	SPOTS %	+ INDIVIDUALS ♀	+ INDIVIDUALS ♂	+ SPOTS
(1) *My/ru h th st p^p sr e^s*	2757	2564	42	24	—	1.2	3837	3495	—
(2) *My/Sb*	980		8	2	2	1.2	—	—	—
(3) *Sb Mw/ru h th st cu sr e^s ca*	1348	1526	46	38	—	2.8	—	—	—
(4) *Mw/Sb*	419		—	—	3	0.7	461		3*
(5) *Mw/ru h th st p^p cu sr e^s*	580	647	3	7	1	0.8	—	—	—
(6) *Mw/h st th sr e^s ro ca*	583	525	4	1	—	0.4	—	—	—
(7) *Mβ/ru h th st sr e^s ca*	1487	1240	6	1	—	0.3	1579	1345	—
(8) *M33j/+*(6 separate experiments)	1915	—	1	—	—	0.05	—	—	—
Total ♀ (excl. exp. (2) (4) (8))	6755	—	110	—	+(?)	1.6	5416	—	—
Total ♂ (excl. exp. (2) (4) (8))	—	6502	—	73	+(?)	1.1	—	4840	—
Grand total	— 16571	—	—	189	—	1.1	10717	—	3

* Spot on ♀: 1.
Spot on ♂: 1.
Spot on sex unknown: 1.
All these had $+^{Sb}$ setae. In one of them the setae were very small in addition.

liminary communication (STERN 1927b) an explanation for these spots was offered which was modeled after the hypothesis of chromosome elimination. A reinterpretation is now necessary in the light of our present knowledge regarding somatic segregation. The experiments to be discussed concern mosaic spots which have occurred in flies heterozygous for the third chromosome dominant Minutes *My*, *Mw*, and *Mβ*. The spots were recognized as areas exhibiting the phenotype of recessive alleles which were present in the zygote in the heterozygous state only. No third chromosome

mutants which are distinguishable in every single micro- or macrochaeta, like the sex-linked mutants *y* and *sn*^{3}, were available but the absence of the dominant Minute is recognizable in a single macrochaeta as is the absence of the dominant gene Stubble (*Sb*, III, 58.2). The recognition of the other genes used depends on the occurrence of multicellular areas in certain regions of the head and thorax.

In comparing the frequencies of autosomal spots (table 19) with those of sex-linked mosaic areas it is necessary to remember the different probabilities of discovering spots which become visible whenever they include a seta and spots which are phenotypically apparent under certain conditions only. It is, however, obvious that the frequency of third chromosome spots is considerably lower than that of comparable sex-linked spots in flies containing *Mn* or the Blond-Minute.

A similar difficulty arises in an evaluation of the frequency of autosomal spots in Minute flies as compared to that of controls which contain no Minute factor. As can be seen from tables 20–24, a large number of spots in Minute flies were recognizable only by the appearance of $+^M$ bristles In not-Minute controls such areas would not be different from the rest of the fly. It is certain that on the whole the presence of autosomal Minutes increases the incidence of autosomal spots as shown by the fact that no spots were found in 7332 not-Minute individuals, controls of experiment 1, nor in 2924 control flies in experiment 4. However, among the *Sb*/+ controls of experiment 4 three individuals occurred each of which possessed one not-Stubble bristle.

Of the three Minute factors studied, the locus of *My* is to the left of the fibre attachment point at 40.4, while that of *My* and *Mβ* is to the right of this point, at 80± and 85.4 respectively.

My. Two experiments involved *My*. In the first, *My* was present in one chromosome, while the following recessives were present in the homologous third chromosome: *ru*, 0.0 roughoid; *h*, 26.5 hairy; *th*, 42.2 thread; *st*, 44.0 scarlet; *p*^{p}, 48.0 peach; *cu*, 50.0 curled; *sr*, 62.0, stripe; *e*^{s}, 70.7, sooty. Phenotypically, such flies show only the Minute bristle condition. Table 20 indicates that 66 spots were found. In all cases (57 out of 66) where the spots enclosed one or more macrochaetae it was seen that these setae were normal in length, indicating the absence of *My*. Whenever the spots covered an area which in a fly homozygous for *h* would exhibit the extra-hair effect of this recessive gene extra hairs were present indicating the absence of the normal allele of *h* (45 cases). In 8 out of 9 cases, where the spot involved part of an eye, the recessive characters roughoid eye-texture and scarlet eye-color were visible, indicating the absence of the normal alleles of these genes. The arista of one antenna was involved 6 times and presented the phenotype of "thread," indicating the absence of

this gene's normal allele. In contrast to the appearance of the phenotypes of *ru*, *h*, *th*, *st*, and $+^{My}$ no spot presented the phenotypes peach eye-color and stripe and sooty body coloration. Peach would have been recognized in the 9 spots which covered part of an eye and sooty or stripe and sooty would have been recognizable in more than 20 spots. The phenotype curled wing did not occur either, but it is unknown if this is an autonomous character which would appear in mosaic spots.

While the foregoing description has taken account of each gene by itself, many spots in reality permitted the determination of the presence or absence of more than one character at the same time. This is to a certain degree obvious from an inspection of table 20. As a whole the findings show that spots produced in *My* flies exhibit all those recessive genes which are located in the $+^{M}$ carrying third chromosome to the left of a point between *st* and *p* and that the spots do not exhibit phenotypes of any genes located to the right of this point. The only exception is represented by one spot which covered part of the head and showed the phenotype *h*, *st*, and $+^{M}$ but not that of *ru*.

TABLE 20

Spots on My/ru h th st p^{p} sr e^{s} flies (cf. table 17).

$h+^{M}$		$ru\ h\ th\ st+^{M}$	$ru\ h\ st+^{M}$	$ru\ st$	$h\ st+^{M}$	$+^{M}$		h	th
1	>1	>2	>2	0	>2	1	>1	0	0
2	32	3	2	3	1	12	5	5	1

The second experiment which involved *My* concerned flies of the constitution *My/Sb*, with the phenotype Minute-y Stubble bristles (table 21).

TABLE 21

Spots on My/Sb flies (cf. table 17).

$+^{M}\ Sb$		$M+^{Sb}$		$(?)M\ Sb$
1	>1	1	>1	1
3	6	2	1	1

There were 13 aberrant areas. In 9 cases the phenotype *My* had disappeared, while that of Stubble was still present, in 3 cases the reverse was true, resulting in Minute, not-Stubble bristles. The 13th spot was formed by one bristle, which showed both Minute and Stubble characters although in abnormal fashion.

Mw. Before we discuss these mosaic spots induced by Minute-y, we shall describe the phenomena in flies possessing Minutes located in the right arm of the third chromosome. The largest experiment involved Minute-w, *Sb*, and the recessives *ru*, *h*, *th*, *st*, *cu*, *sr*, e^{s}, *ca* (*ca*, III, 100.7

claret; table 22: *Sb Mw/ru h th st cu sr e^s ca*). In 73 out of the total of 84 spots both *Sb* amd *Mw* had disappeared. Of these 73 spots 14 were large enough and were situated in suitable regions so that the presence or absence of e^s or e^s and *sr* was recognizable; indeed, these genes, originally present in heterozygous condition only, were phenotypically obvious in all 14 cases, as well as in 4 more areas (not included in the 73 areas) which did not involve macrochaetae, so that their nature in respect to *Sb* and *Mw* could not be tested. Two $+^{Sb}+^{M}$ spots involved part of the eye and were claret-colored. No single spot exhibited the phenotypes *ru*, *h*, *th*, or *st*, although *ru* and *st* would have been recognizable in 2 spots, *h* in at least 9 spots, and *th* in 1 spot. In total, 73+4=77 spots can be interpreted as exhibiting when possible the phenotypes of all recessive genes located in the $+^{M}$ carrying third chromosome to the right of a point between *st* and

TABLE 22

Spots on Sb Mw/ru h th st cu sr e^s ca flies (cf. table 17).

$+^{Sb}+^{M}$ e^s(or e^s *sr*)	e^s or e^s *sr*	$+^{Sb}+^{M}$ *ca*	$+^{Sb}+^{M}$		$Sb+^{M}$ *ca*	$Sb+^{M}$		$+^{Sb}$ *M*
	0	>1	1	>1	>1	1	>1	1
14	4	2	2	55	1	2	2	2

Sb, and they do not exhibit phenotypes of any genes located to the left of this spot. These findings are completely opposite to the findings regarding *My*.

There occurred 7 exceptional spots. They agreed with the rest by not exhibiting any genes located to the left of the region noted (1 case: $+^{ru}+^{h}$), but differed by showing either $+^{Sb}M$ or $Sb+^{M}$ bristles and in addition neither *sr* nor e^s (1 case).

A corroboration of these results is provided by 11 spots in flies of the constitution *Mw/ru h th st p^p cu sr e^s* and 5 spots in flies of the constitution *Mw/h th st sr e^s ru ca* (table 23). In 13 cases the recessive genes of the right half of the $+^{M}$ third chromosome were visible ($+^{M}$, e^s, or *sr*, e^s according to the region involved). In 6 out of these 13 cases the phenotypic absence of the left arm gene *h* was evident although its presence would have been recognizable. While these results agree with the general deduction which has just been made, the remaining 3 mosaic areas were exceptional again. They did not exhibit the phenotypes *sr* and e^s, in spite of location on suitable thorax regions. In two of these spots it could be ascertained that *h* did not appear. This finding agrees with that in all other spots.

A last experiment involving *Mw* concerned flies of the constitution *Sb/Mw* (419 individuals). Only 3 spots were found, two of which were phenotypically $Sb+^{M}$ (1 doubtful), the third being $+^{Sb}M$.

An experiment in which *M* β was present is reported in table 24. Of a

TABLE 23

Spots on Mw flies (cf. table 17).

CONSTITUTION	$+^M$ e^s (or e^s sr) 0	$+^M$ e^s (or e^s sr) >2	e^s or e^s sr 0	$+^M$ 1	$+^M$ >1	$+^M+^{e,sr}$ >1
Mw/ru h th st p^p *cu sr* e^s	3	—	4	1	1	2
Mw/h th st sr e^s *ro ca*	1	2	—	1	—	1

total of 7 spots 6 exhibited suitable recessive genes located in the right arm of the not-Minute third chromosome, while no spot with genes located in the left arm appeared (1 spot was definitely $+^h$). The seventh mosaic area was exceptional in having $+^M$ bristles but also $+^{sr}$, $+^e$ coloration (*h* did not appear either).

All findings regarding autosomal mosaic areas in flies carrying third chromosome Minutes can be summarized in the following statements:

(1) Spots exhibit only phenotypes of recessive genes, which are located in one arm of the not-Minute carrying chromosome. If the Minute factor is in the left arm of the third chromosome (*My*), only recessive genes in the left arm become visible; if the Minute factor is located in the right arm (*Mw*, Mβ), only recessive genes in the right arm exhibit their effect.

(2) In the majority of spots all genes located in the respective arm are involved.

(3) A smaller number of spots exhibits only the phenotypes of *some* genes located in one arm.

Originally these findings were explained by the elimination assumption. That arm of the Minute-bearing chromosome or part of it in which the Minute is located was thought to be broken off and lost from the cell. The following considerations make this assumption untenable now: (a) the one spot reported in table 20, which exhibited *h*, *st*, and $+^M$, but not *ru* can be explained by elimination only if one assumes the loss not of a whole arm or a whole end section of the chromosome but of a middle piece. Such a process would necessitate special assumptions with regard to elimination which seem rather artificial. It should be mentioned, however, that a slight possibility exists that the absence of the phenotype *ru* in the eye-spot may not really indicate the presence of the dominant $+^{ru}$ but may conceivably be an extreme phenotypic variation of the somewhat variable expression

TABLE 24

Spots in Mβ/ru h th st cu e^s *ca flies (cf. table 19).*

$+^M$ e^s (or e^s sr)	e^s or e^s sr	$+^M$	$+^M+^{e,sr}$ ($+^h$)
2	3	1	1

of *ru*. (b) As will be shown below, the occurrence of autosomal spots can be interpreted by the theory of somatic crossing over in autosomes. As the theory of somatic crossing over has been proven to be superior in the case of sex-linked mosaics, it is a priori probable that it holds also for autosomes. A direct test which would be provided by a demonstration of autosomal twin spots cannot be given, since autosomal spots occur hardly at all in not-Minute flies, while in Minute flies one of the segregation products after somatic crossing over becomes homozygous for the Minute factor and is not viable. Thus only a single, not a twin, mosaic area can appear.

Granting the validity of the theory of autosomal somatic crossing over and segregation, a detailed analysis of the data yields an unexpected result. We consider first those cases which form the majority and which demonstrate that the region of somatic crossing over is restricted to the neighborhood of the spindle fibre point. This point in the third chromosome is located in the middle, between scarlet (44.0) and pink (48.0, peach is an allele of pink). It was just this interval in experiment 1 (table 20) which divided the genes whose phenotypes appear in mosaic areas from those which did not appear; in experiment 3 (table 22) where the critical region could not be narrowed down as completely, but could only be said to lie within the interval *st* (44.0) to *Sb* (58.2) the spindle fibre point is again included. Restricting our analysis to single crossovers between two of four strands only, there are four possible crossing over and segregation processes which lead to the appearance of somatic areas:

(1) *Crossing over to the left of the fibre point.*

(x) If *A B* S′ *C D/a b* S² *c d* is the original constitution of the autosomes, S′ and S² representing homologous fibre points, segregation of the four chromatids after crossing over with normal equational separation ot the daughter fibres **(x)** yie'ds: *A B* S′ *C D/A B* S² *c d* and *a b* S′ *C D/a b* S² *c d*. The atter constitution would be visible as a mosaic area exhibiting *a* and *b*.

(y) Non-disjunctional separation of the daughter fibres **(y)** yields *A B* S′ *C D/a b* S′ *C D* and *A B* S² *c d/a b* S² *c d*, the latter exhibiting *c* and *d*.

(2) *Crossing over to the right of the fibre point.*

(x) segregation results in *A B* S′ *C D/a b* S² *C D* and *A B* S′ *c d/a b* S² *c d*, yielding a spot *c d*.

(y) segregation results in *A B* S′ *C D/A B* S′ *c d* and *a b* S² *C D/a b* S² *c d*, yielding a spot *a b*.

An *a b* spot therefore occurs either as a consequence of crossing over to the left of the fibre point and equational fibre segregation (process **1x**) or as a consequence of crossing over to the right and non-disjunctional separation (process **2y**). Correspondingly a spot *c d* occurs either in conse-

quence of crossing over to the right of the fibre point and equational fibre segregation (process **2x**) or after crossing over to the left of the fibre point and non-disjunctional separation (process **1y**). It is believed that only one of each alternative is actually realized and that this involves normal equational separation of daughter fibre points. Reasons for this assumption are (1) the analogy with somatic segregation of X chromosomes in which equational separation has been demonstrated to be the rule and (2) the demonstration to be given in the next paragraph that equational separation of fibre points is the only method followed after somatic crossing over distal to the fibre region in the third chromosome.

An analysis of the smaller group of spots which exhibit only the phenotypes of some genes located in one third chromosome arm leads to an interpretation which considers these areas as caused by consequence of crossing over in regions other than that including the spindle fibre point. Single crossovers are sufficient to yield the different spots reported in columns 6, 7 and 8 of table 22, and the last columns of tables 23 and 25. All these spots occurred in experiments which involved Minute factors in the right arm of the chromosome. The locus of crossing over can be placed to the right of *e* (70.7) and to the left of *Mw* (80.0) or *Mβ* (85.4) respectively; this is judged from the fact that all recessive loci to the left of the *e-M* region failed to appear in mosaic areas, while those to the right did appear. Non-disjunctional segregation would have yielded areas which exhibit all recessive genes located to the left of the crossover point, thus including loci from both arms of the chromosome. But no such areas did occur. It can be demonstrated that these cases of somatic crossing over can have been followed only by equational fibre point segregation which would lead to the observed types of spots.

There remains a discussion of the few spots not yet dealt with. If the one *h st*$+^{M}$ spot of table 20 can be regarded as genotypically $+^{ru}$ as indicated by its phenotype, a case of double crossing over would be represented, the crossover points being located in the regions *ru-h* and *st-p*. A double crossover between the fibre point and *Sb* and between *Sb* and *Mw* would explain the $+^{Sb}Mw$ spots of table 22 and the $+^{Sb}Mw$ spot mentioned in the last paragraph of the section on *Mw*. The *My*$+^{Sb}$ areas recorded in table 21 can be regarded as crossover products to the right of the fibre point. Such spontaneous crossovers are believed also to be the explanation of the occasional occurrence of $+^{Sb}$ spots in not-Minute flies (table 19 (4), control). An alternative explanation assuming crossing over to the left of the fibre point would demand non-disjunctional fibre segregation. For the exceptional spot recorded in the last column of table 21 and the $+^{Sb}$ *M*-like spot among the controls of experiment 4, table

19, no discussion of hypothetical interpretations seems warranted.

The unexpected result brought out in the analysis of areas involving third chromosome genes consists in the definite correlation between the location of the Minute factor in the left or right arm of the chromosome and the corresponding location of the crossover point. No well-founded theory of this relation can be given.

In addition, it seems noteworthy that the distribution of crossovers along the chromosome is greatly changed as compared to germinal crossing over. The great majority of somatic crossovers seems to fall within the interval *st-p* which comprises less than four per cent of all germinal crossovers. As this interval encloses the fibre attachment point it represents the region adjacent to this point. The concentration of somatic crossovers in these regions parallels our former findings concerning the massing of somatic crossovers in the region adjacent to the fibre point of the X chromosome.

The occurrence of autosomal crossing over in females and males

Autosomal mosaic areas appeared both on females and on males. Somatic crossing over in autosomes is thus shown to occur in both sexes. However, it is seen from table 19 that a probably significant lower frequency was found in the males.

It may be added that an experiment was made to test the question whether an autosomal Minute would induce crossing over in its chromosome in the germ cells of males. Males of the constitution *Mw/h st cu, sr, e^s ca* were backcrossed to homozygous *h st cu sr e^s ca* females. None of the 4151 F_1 individuals were crossovers (class *Mw* = 2092, class *h st cu sr e^s ca* = 2059). There is then either no effect of *Mw* or the effect is too small to become apparent in this sample of more than 4000 flies. The experiments on induction of crossing over in the male of *Drosophila melanogaster* by FRIESEN (1934), PATTERSON and SUCHE (1934) and A. F. SHULL and WHITTINGHILL (1934) by means of X-rays or heat yielded positive results with much smaller numbers.

MOSAIC AREAS IN FLIES HETEROZYGOUS FOR X CHROMOSOME INVERSIONS

Whenever an X chromosome was present in our experiments which carried the bobbed-deficiency, described by SIVERTZEV-DOBZHANSKY and DOBZHANSKY (1933), unexpected peculiarities relating to mosaic areas were found. Attempts at interpretation met difficulties until it became known that the *bb^{D1}* chromosome, besides lacking a section of a normal X chromosome, carries a long inversion with the end points at 9± and

TABLE 25a

Spots in experiments involving primarily y, sn^3, and bb^{Df}. No Y chromosome present.

EXP.	CONSTITUTION*	IND.	SPOTS	NO SPOTS	sn^3 1	sn^3 2	sn^3 >2	y 1	y 2	y >2	$y\ sn^3$ 1	$y\ sn^3$ 2	$y\ sn^3$ >2	y-sn^3 2	y-sn^3 >2	y-sn^3 y 2	$+\sigma^3$ COL.
1a	$y\ bb^{Df}/sn^3$	884	12†		6	—	2	4	—	—							
		340	28		8	2	—	12	3	3							
		683	153		34	8	11	62	16	10				4	7		1
		122	18		2	—	1	7	3	2				2	—		1
					50	10	14	85	22	15				6	7		
Totals Exp. 1		**2029**	**211**	**10**	**74**			**122**			—			**13**			**2**
2	$y\ sn^3\ bb^{Df}/+$				2	—	—	6	3	3	19	1	1			1	1
		838	37	4	2			12			21			—		1	1
					23‡	1(?)	—	14	4	2	—			—		—	—
3a	$sn^3\ bb^{Df}/y$	821	44		24			20									
		22	20		3	1	—	8	4	—	—			3	1	—	—
					4			12						4			
		29	6		2	—	1	—	1	1	—			—	1	—	—
					3			2						1			
				8§	28	2	1	22	9	3				3	2		
Totals Exp. 3		**872**	**70**	**(6)§**	**31**			**34**						**5**			
4	$bb^{Df}/y\ sn^3$	740	15	2	1	—	(1?)	3	2	1	1	1	4	—	—	—	1
					2			6			6						

* Besides the genes indicated, other recessive sex-linked factors were present in heterozygous condition in some experiments. An autosomal Minute was present in some of the flies of exps. 1a, b, c. In exp. 3c and partly in exp. 4 the constitution in respect to the *bb* locus was bb^{Df}/bb.

† Inspected for head and thorax spots only.

‡ Two doubtful.

§ The value of 6% is obtained if one excludes exp. 3b which represents individuals of one single culture which, raised at the same time with 29 cultures recorded as exp. 3a, gave an exceptionally high number of *y* spots.

64± (BEADLE and STURTEVANT 1935). On the basis of this fact an explanation of the observed spots could be given. Furthermore, in the course of working with flies containing bb^{Df} it was found that bb^{Df} stocks frequently contained supernumerary Y chromosomes as had been described for an independent bb^{Df} stock by GERSHENSON (1933). This led to the discovery of a striking influence of the Y chromosome on somatic crossing over. Accordingly the results for each experiment involving a known constitution of the X chromosomes will be given separately for those cases in which no Y chromosome was present in the females and those in which a Y chromsome was present. Special crosses had to be undertaken in order to assure the absence or the presence of an extra Y chromosome and in many cases progeny tests were carried out as independent tests.

Experiments involving y, sn^3, and bb^{Df}; no Y chromosome present

As in foregoing analyses, all four possible combinations of the three allelic pairs were secured so that any interpretation has to stand a fourfold test. The results are given in tables 25a and b. If we compare them with the similar experiments involving *y* and sn^3 recorded in tables 5 and 8 we note (a) the relatively low number of spots with 2 or more setae, (b) the relatively low number of twin spots in experiments 1 and 3, (c) the relatively high number of spots other than *y* sn^3 in experiments 2 and 4. A further very striking fact is not indicated in table 25: although the setae in non-mosaic areas were of normal size, the majority of *y* sn^3 setae in experiment 2 and of the sn^3 setae in experiment 3 were of very small size varying from fine, thin structures to larger but still distinctly subnormal ones. A similar picture was presented by many *y* setae in experiment 1. It was different with at least part of the *y* setae in experiments 2 and 3, as well as with most of the different setae in experiment 4; they were of normal size.

The explanation of these phenomena is based on MCCLINTOCK'S (1933) cytological findings with reference to crossing over involving chromosome inversions. Her results have been confirmed by later workers (MÜNTZING 1934; SMITH 1935; MATHER 1935; HÅKANSSON 1936) and have been applied to a genetic analysis by BEADLE and STURTEVANT (1935). We shall restrict our discussion to the consideration of single crossovers and equational fibre point segregation, processes which can account for the great majority of spots. Crossing over can occur in two different sections of the X chromosome, outside the inverted region and inside. The first section in a chromosome carrying the bb^{Df} inversion is formed by the left end of the X chromosome, from *y* to about the locus 9, the second section by the rest of the chromosome. Whenever somatic crossing over occurs between the left ends of the chromosomes we obtain the same results as discussed

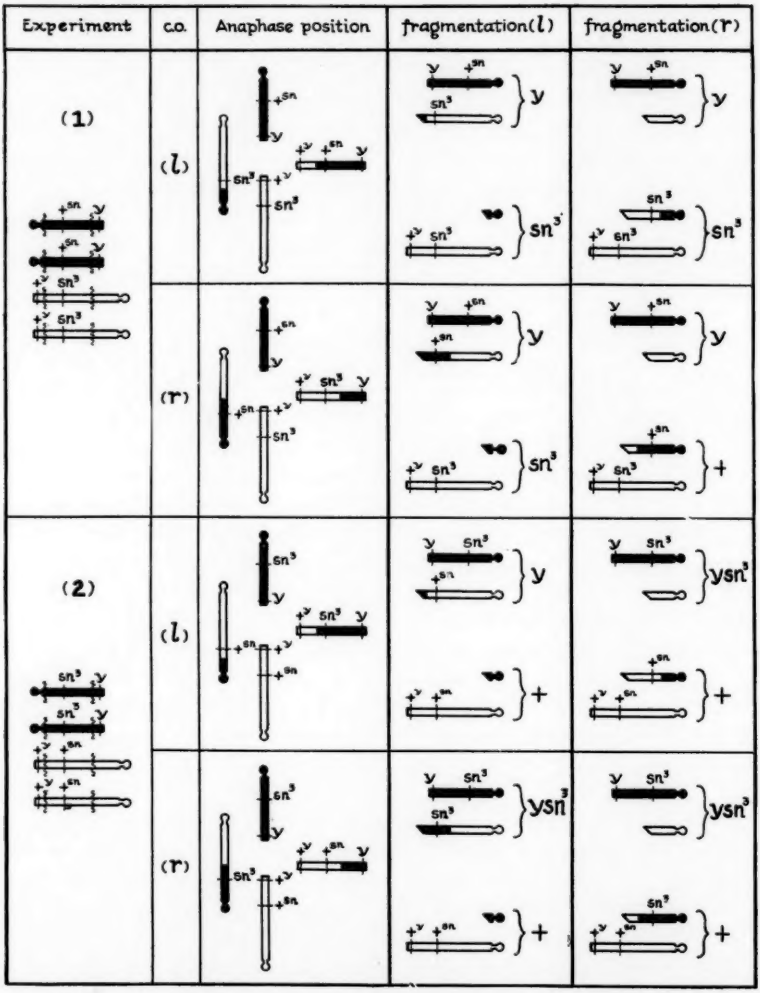


FIGURE 10 a, b. Four experiments involving *y*, *sn*^{3} and *bb*^{Df}. (See tables 25–27, experiments 1, 2, 3, 4.) The results of **x**-segregation after crossing over to the left (l) and to the right (**r**) of *sn*^{3} and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (**r**) of *sn*^{3}. The lines } indicate the end points of the inverted region.

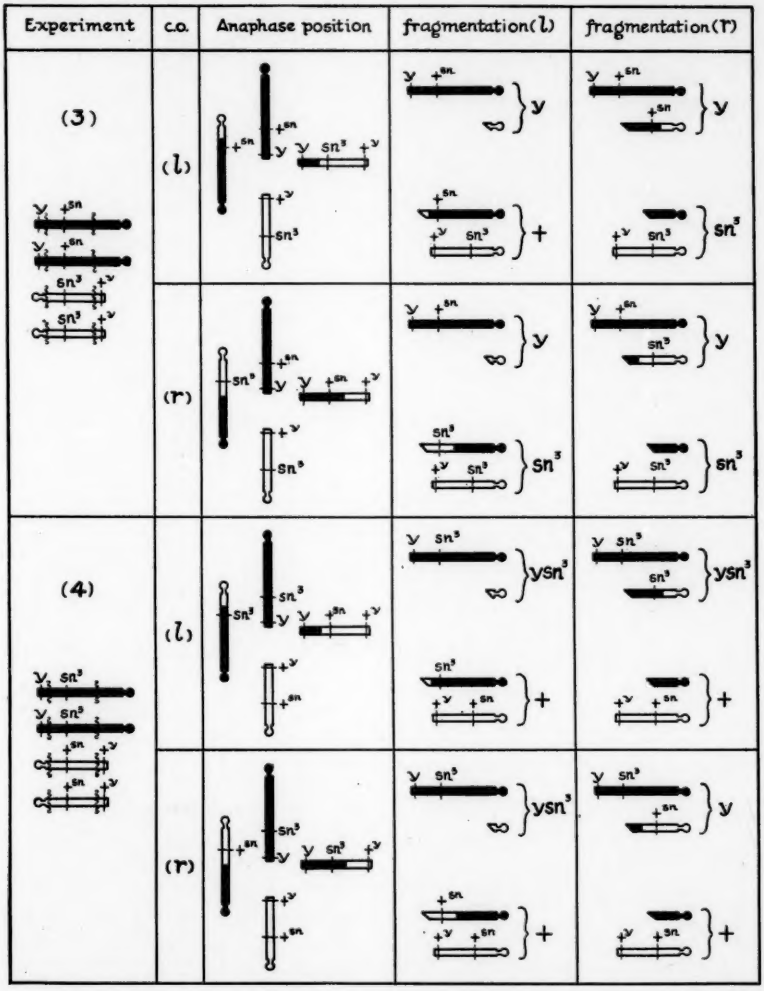


FIGURE 10b (see opposite)

in an earlier chapter, namely, in all four experiments, the appearance of single *y* spots. The spots contain two X chromosomes and the phenotype of these *y* setae is expected to be normal. Part of the *y* spots with setae of normal length are believed to have originated in this way.

A very different situation is produced as a consequence of somatic crossing over within the inverted region (fig. 10). If crossing over occurs at a four strand stage, two normal non-crossover strands will be formed, while the two other strands will yield one strand containing no spindle fibre point and one containing one fibre point on each end (fig. 10). If segregation is accomplished by equational disjunction of sister fibre points, two possible distributions result.

TABLE 25b

Sexual coloration of critical spots from table 25a.

EXP.	*sn*³		*y*		*y sn*³	+	TOTAL	
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.
1b	1	—	—	—	—	—	15	3
c	5	2	9	—	—	1		
2	—	—	1	—	—	1	1	1
4	—	—	1	—	1	—	1	1
Totals							17	5

(1) Segregation **(z).** Both non-crossover strands go to one pole and the crossover products to the other pole. The chromatid without any attachment is expected to be eliminated. In this case one cell obtains a constitution not different from that of any non-crossover cell, while the other cell will receive the chromatid with two attachment points. This cell will lack completely the left end of the X chromosome and presumably would not give rise to a viable product. Thus, segregation **(z)** would not yield visible mosaic areas.

(2) Segregation **(x).** In this case the two non-crossover chromatids segregate to opposite poles, while the chromatid with two attachment points becomes oriented parallel to the spindle axis in such a way that sister fibre points are directed toward opposite poles. The chromatid without any fibre point is again regarded as being left in the middle of the mitotic figure and thus eliminated. There are two ways by which such a process might result in two daughter nuclei.

First, it is possible that the double-fibre-point chromatid will not be included in the nuclei and so be eliminated. In this case cel s with the following constitutions will be formed:

In experiment (1) twin cells with *y* bb^{Df} and sn^3
" " (2) " " " *y* $sn^3 bb^{Df}$ and +
" " (3) " " " sn^3 bb^{Df} and *y*
" " (4) " " " bb^{Df} and *y* sn^3

In all cases only one X chromosome will be present in each nucleus. The cells with bb^{Df} are not expected to survive. Thus single mosaic areas would be formed of the constitutions sn^3, +, *y*, or *y* sn^3. Actually a process of elimination cannot account for more than a very small number of spots, at best. For it does not account at all (a′) for the abnormally small size of setae in the majority of spots, (b′) for the $+^{sn}$ spots in experiment 1 (125 out of 209), (c′) for none except 1 spot (out of 37) in experiment 2, (d′) for the $+^{y}$ spots in experiment 3 (36 out of 70), (e′) for the not-*y* sn^3 spots in experiment 4 (9 out of 15), and (f) for the fact (table 25b) that out of a total of 22 spots in which the sex could be determined only 5 showed male coloration whereas the elimination process should lead to male spots only. The same arguments dispose also of the possibility that the two-fibre-point chromatid as a whole often becomes included in one of the daughter nuclei. In such cases the sister nucleus would have a 1X constitution as outlined above.

Let us therefore consider a second possibility. Again we regard the case that the two non-crossover chromatids go to opposite poles and that the two-fibre-point chromatid is arranged so that sister fibre points of the chromosome group segregate equationally. The two-fibre chromatid will then be subjected to conflicting forces, one end with its fibre attachment "pulling" in the opposite direction from the other. On the basis of the cytological observations of the authors named above, who studied two-fibre chromatids in meiosis we assume that the two-fibre chromatid breaks at some point under the stress and that the two fragments become included, each in one daughter nucleus. Such a hypothesis leaves room for two pairs of alternative possibilities resulting in four types of areas to be expected (fig. 10). The two-fibre chromatid will either contain sn^3 or $+^{sn}$ according to the region in which crossing over occurs in the inverted section, namely to the left of sn^3 (as measured in the non-inverted chromosome) or to the right of it. Also, the fragmentation break can fall on either side of the locus of sn^3. The consequences of these four possible occurrences are given in figure 10. Sister cells originate in each case which possess one complete X chromosome (disregarding the deficiency for *bb*) and one X fragment of varying length.

No prediction can be made as to the relative frequencies of the four processes. Crossing over to the left of sn^3 can occur in the section from locus 9–21 only, while crossing over to the right can occur between the loci 21 and 64. This fact would seem strongly in favor of crossing over to

the right of the sn^3 locus. But as both the region to the left and to the right of sn^3 extends up to a fibre point in one of the two chromosomes the incidence of crossing over on the two sides of sn^3 might tend to become more equal. The presence of the $bb^{D/}$ introduces another factor of unknown influence. With regard to the fragmentation break it may seem that it should preferably fall to the "right" of the sn^3 locus (measured in comparison to the non-inverted chromosome), the more so as the "right" section is not only so much longer than the "left" one but includes in addition to the active region the long inert part which on account of the *bb*-deficiency is absent on the other side of sn^3. However, no a priori reasoning should be applied to this situation because the effect of the inert material on the mechanical properties of the X chromosome is an open question since SCHULTZ's (1936) and OFFERMANN's (1936) discovery of visible cytological differences in X chromosomes lying in salivary gland nuclei with or without extra inert material.

If we want to compare our actual findings with the expectations from the proposed scheme we must call attention to one more point. All nuclei originating from the different processes possess unbalanced X-chromosomal constitutions. Each contains only once the normal left end of the X chromosome, from *y* to the beginning of the inversion, but each contains certain other regions in duplicated condition, once in the non-crossover chromatid and once in the chromosome fragment. The degree of unbalance is variable according to the breakage point of the fragmenting chromatid. *Zygotes* with such unbalanced constitution will, in most cases, not be able to give rise to viable individuals, but it is permissible to assume a certain degree of viability of *hypodermal areas*. This leads us to expect mosaic areas originating from these processes to be small in size and covered with setae of varying degrees of abnormal growth. In many cases it is to be anticipated that the viability of two sister cells is different so that only one will be able to give rise to a mosaic area. Three striking phenomena regarding table 25a are seen at once to be in agreement with such expectations, namely the facts (a) "relatively low number of spots with 2 or more setae in experiment 2 and 4," the fact (b) "relatively low number of twin spots in experiments 1 and 3," and the finding that the majority of setae were abnormally small. Furthermore the genetic type of spots found in each of the four experiments agrees with the expectations (fig. 10). Only very few spots occur which cannot be accounted for on the assumption of single crossing over within the inversion and fragmentation of the two-fibre-point chromatid; but these spots are to be expected from crossing over outside of the inversion and from multiple crossovers. It cannot be stated with certainty whether the four theoretical possibilities

concerning the region of crossing over and of fragmentation are all realized. Experiment 2 indicates that the great majority of spots, the *y sn*^{3} spots, originated as a result of one or all of the processes "crossing over to the left of *sn*^{3}, fragmentation break to the right of *sn*^{3}" **(l,r),** "crossing over right, fragmentation left" **(r,l),** and "crossing over right, fragmentation right" **(r,r).** The *y* spots with small setae, which occurred in the same experiment, point to the occurrence of the fourth process "crossing over left, fragmentation left" **(l,l).** In experiment 4 the presence of *y* spots seems to point to the occurrence of process **r,r.** If then we take the representation of processes **l,l** and **r,r** for granted, we have established the position of crossover and of fragmentation points to both sides of *sn*^{3} in two combinations. There seems no objection to the assumption that the two other combinations, **l,r** and **r,l** are also realized. An attempt to evaluate the frequency of the four types on the basis of the actual data fails due to the variability of the results.

The total frequency of spots is higher in experiments 1 and 3, namely 10 per cent and 8 (or 6) per cent, respectively, as compared to experiments 2 and 4 where it is 4 and 2 per cent. This agrees in a general way with the fact (fig. 10) that in experiments 1 and 3, 7 out of the 8 segregational constitutions can potentially give rise to visible mosaic areas, while in experiments 1 and 4 only 4 out of the 8 constitutions can lead to visible spots.

Table 25b showed that 17 out of 22 spots in which the sex could be recognized were female and the remaining 5 were male. In these experiments both types of spots are to be expected, for a very short fragment in addition to the one X chromatid should result in a male spot and the presence of a longer fragment in a female spot.

Summary: The hypothesis of somatic crossing over in a heterozygous inversion accounts for the observed mosaic spots if it is assumed that the resulting chromatid without any fibre point is eliminated and the chromatid with two fibre points is fragmented so that each of the two fragments with its fibre point is included in a daughter nucleus.

Experiments involving y, sn^3*, and bb*^{D1}*; a Y chromosome present*

Females with essentially the same constitution in regard to X-chromosomal genes as those reported in the preceding chapter, but possessing a Y chromosome, were obtained and exhibited the spots summarized in tables 26a, b. A comparison of the two groups, without and with Y chromosome (table 27) yields three results:

(a) The presence of a Y chromosome increases the frequency of spotting. The percentages for the four pairs of experiments are:

TABLE 26a

Spots in experiments involving primarily y, sn^3 and bb^{Df}. A Y chromosome was present.

EXP.	CONST.*	IND.	SPOTS	% SPOTS	sn^3 1	sn^3 2	sn^3 >2	y 1	y 2	y >2	y-sn^3 1	y-sn^3 2	y-sn^3 >2	y-sn^3 2	y-sn^3 >2	y-$y sn^3$ 2	y-$y sn^3$ >2
1a	$y\ bb^{Df}/sn^3$ Y	161	62	—	3	1	3	36	12	7							
b		19	9	—	—	—	—	3	1	5							
					3	1	3	39	13	12							
Total		**180**	**71**	**39**	**7**			**64**									
2	$y\ sn^3\ bb^{Df}/+$Y	507	145	29	5(1?)	1	—	11(1?)	3	—	53	36	34	—		1	1
Total					**6**			**14**			**123**					**2**	
3	$sn^3\ bb^{Df}/y$ Y	458	57	12	34(3?)	1	2	10	2	5	—			2	1	—	
Total					**37**			**17**						**3**			
4	$bb^{Df}/y\ sn^3$ Y	863	50	6	10	3	3	12	5	10	4	—	3	—		—	
Total					**16**			**27**			**7**						

* All flies in exp. 1a contained an autosomal Minute. In exp. 1b and 4 the constitution in regard to the *bb*-locus was bb^{Df}/bb.

Experiment	*Without Y*	*With Y*
1	10	39
2	4	29
3	8 (or 6)	12
4	2	6

(b) The relative frequencies of different types of spots are changed by the presence of a Y chromosome.

(c) No phenotypes of spots in flies carrying a Y chromosome occur which did not also occur in flies without a Y chromosome.

A closer examination shows that the increase of total frequency of spots under the influence of a Y chromosome is mainly due to the rise of one particular class in each experiment. The classes with the heightened frequency are in experiment 1: *y*; in 2: *y sn*^{3}; in 3: *sn*^{3}. In experiment 4 two classes, *sn*^{3} and *y*, have been increased.

TABLE 26b

Sexual coloration of critical spots from table 26a.

EXP.	*sn*^{3} ♂ COL.	*y* NO ♂ COL.	*y sn*^{3} NO ♂ COL.	*y sn*^{3} ♂ COL.	TOTAL NO ♂ COL.	TOTAL ♂ COL.
1b	—	1*	—	—	1	0
2	—	—	27(1?)	1	27	1
3	1	1	—	—	1	1
4	—	6	1	1	7	1
Total					**36**	**3**

* This spot comprised a large part of the abdomen.

The data can be accounted for by the hypothesis that the presence of a Y chromosome leads to a higher frequency of the crossing over and fragmentation process **r,r** (fig. 10). This is obvious in experiments 1 and 2 where only *y* and *y sn*^{3} spots will become more numerous. In experiment 3 where the process **r,r** results in *y* and *sn*^{3} sister cells the further assumption is necessary that the viability of the *y* segregate is so much lower than that of the *sn*^{3} segregate as to result mainly in *sn*^{3} single spots. This assumption is compatible with the data in experiments 1 and 2. In experiment 4 the hypothesis leads to the expectation of an increase in + segregates which are not recognizable. According to this the higher frequency of *sn*^{3} and *y* spots in 4 has to be accounted for in a different way. No explanation for this increase will be advanced as the rather low numbers would make any attempt unsafe. But it may be pointed out that apart from the very striking increase of the classes *y* and *sn*^{3} in experiments 1 and 2, a slight increase is visible also in the percentage of some other spots.

The spots occurring in experiments with the Y chromosome are larger than in those without a Y chromosome (tables 25a and 26a). This is reflected especially in the fact that 70 out of 123 *y sn*^{3} spots in experiment 1, table 26a, covered 2 or more setae. The significance of this result is doubtful.

The interpretation of the influence of a Y chromosome on somatic crossing over by means of a preferential increase in process **r,r** leaves still open the question as to the underlying mechanism. The occurrence of segregational products in connection with the process **r,r** is dependent on three conditions: (1) crossing over to the right of *sn*^{3} and (2) fragmentation to the "right" of *sn*^{3}. Both conditions must be involved in causing the increase

TABLE 27

Summary of spots from experiments involving primarily y, sn^3*, and bb*^{Df}*, without or with the presence of a Y chromosome. Details in tables 25, 26.*

EXP.	CONST.	IND.	SPOTS	% SPOTS	*sn*^{3}	*y*	*y sn*^{3}	*y-sn*^{3}	*y-y sn*^{3}	♂ COL. +
1	*y bb*^{Df}/*sn*^{3}	2029	211	10	74	122	—	13	—	2
	y bb^{Df}/*sn*^{3} Y	180	71	39	7	64	—	—	—	—
2	*y sn*^{3} *bb*^{Df}/+	838	37	4	2	12	21	—	1	1
	y sn^3 *bb*^{Df}/+Y	507	145	29	6	14	123	—	2	—
3	*sn*^{3} *bb*^{Df}/*y*	872	70	8(6)	31	34	—	5	—	—
	sn^3 *bb*^{Df}/*y* Y	458	57	12	37	17	—	3	—	—
4	*bb*^{Df}/*y sn*^{3}	740	15	2	2	6	6	—	—	1
	bb^{Df}/*y sn*^{3} Y	863	50	6	16	27	7	—	—	—

of the process **r,r**: higher frequency of condition 1 can be effective only if accompanied by higher frequency of 2. A rise of 2 by itself would not be compatible with the observed total increase of spots as it would mean only an increase in number of **r,r** processes at the cost of a decline of **r,l**. The result of these deductions is the hypothesis that the influence of a Y chromosome on the frequency of certain spots is accomplished by an increase in the frequency of somatic crossing over to the right of *sn*^{3} (in the normal X chromosome) combined with an increase in fragmentation frequency in that part of the two-fibre-point chromatid which lies between *sn*^{3} and the normal (not-*bb*^{Df}) end of the chromatid. A third element, a possible rise in the frequency of **x**-segregation at the expense of **z**-segregation can at best be only an additional factor. Further data regarding the influence of a Y chromosome will be discussed in following chapters.

An exceptional case of segregation in experiments involving y, sn³, bb^Df and an extra Y chromosome

All fourteen experiments recorded in tables 25a and 26a gave results which agreed among each other. A fifteenth experiment, however, had a fundamentally different outcome. The flies in this case were of the same constitution as those in "experiment 1 with Y," namely *y bb^Df/sn³*; Y. They also contained the autosomal Minute 33j in heterozygous constitution, but so did many of the females of experiment 1a, table 26a, first line. Table 28 records the results which were obtained from a set of 7 cultures

TABLE 28

Spots from an exceptional experiment involving y, sn³, bb^Df and a Y chromosome.

IND.	SPOTS	%	sn^3 2	y 1	y 2	y >2	$y\text{-}sn^3$ >2	$y\text{-}sn^3$ >2	SEX OF SPOTS (ALL y)* NO ♂ COL.	♂ COL.
146	277	191	1	98	59	115†	3	1(?)	—	—
—	—	—	1		272		3	1(?)	4	33‡

* In addition: 1 spot of the *y-sn³* twin spots showed ♀ coloration in the *sn³* part.

† 1 spot possibly belongs to the *y-sn³* twin spot class.

‡ 1 spot which covered the 6th right tergite had ♀ coloration laterally but not toward the median.

with two or three female and three male parents each. They were started several months apart from the experiments of table 26a.

The results deviate in three ways from those discussed in the last chapter: (a) 272 out of 277 spots belong to only one class; (b) the setae in spots were of normal size; (c) 33 out of 37 *y* spots were of male constitution.

It is unknown in which respect the flies in this experiment deviated fundamentally from those discussed above. That they contained a *y bb^Df* and a *sn³* X chromosome as well as a Y chromosome is certain, both from the parental constitutions and from progeny tests of 10 individuals. Although it has not yet been possible to duplicate the results they have been presented here as the numbers involved leave no doubt as to their significance. Besides, it is possible to account for them by the following assumptions: if, for reasons unknown, pairing between the genetically active regions of the two X chromosomes was inhibited and if pairing and somatic crossing over between the homologous regions of the X and Y occurred, then only the *sn³* bearing X and the Y would be involved. This is because the *bb^Df* chromosome lacks the region which finds its homologue in the Y chromosome. Equational segregation of the three pairs of fibre points will result in a *y bb^Df* chromatid going to each pole and in addition, in case of x-segregation of the crossover tetrad, in two *sn³* X chromatids going to one pole

and two Y chromatids going to the other pole (fig. 11). The result will be 3X and 1X sister cells. The 3X cells and their descendants do not form visibly mosaic areas on account of their normal phenotype, while the 1X cells form *y* areas. The size of setae in these areas is normal, as the deficiency for *bb* in the X chromosome is "covered" by the Y chromosomes and no extra X chromosome fragment is present. The sex of *y* spots, if recognizable, will be male. It is obvious that the hypothesis fits most of the facts but it should be remembered that no independent tests of it are available at present. Spots other than *y* with male constitution may

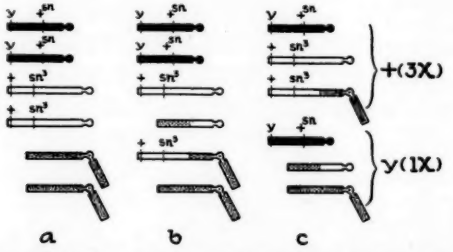


FIGURE 11. An hypothesis to account for the exceptional results in an experiment with *y* bb^{Df}/sn^3; Y flies. a. The six chromatids (2 bb^{Df}; 2 normal X; 2 Y). b. Four non-crossover chromatids and two chromatids resulting from crossing over between one normal X chromatid and one Y chromatid. c. The result of **x**-segregation of the six chromatids.

be thought to have originated in consequence of occasional crossing over between the X chromosomes as discussed in the foregoing chapter.

Experiments involving y, sn^3, bb^{Df} and Theta; no Y chromosome present

The hypothesis of the formation of a chromatid with two fibre points through crossing over within the inverted section, and the subsequent fragmentation of this chromatid, provided an explanation of the observed mosaic areas. It may, however, be asked if the differences in types of spots which occurred in the experiments with bb^{Df} as compared with those in which no inversion was involved were really great enough to warrant the elaborate scheme presented. Although the evidence presented seems to be in agreement with the hypothesis, further data will be adduced in the following sections which strengthen it greatly.

An analysis of the data given in table 29a, experiments 1 and 2 might profitably start with a comparison of the types and relative frequencies of spots in the experiments tabulated in table 13. There as here *y*, sn^3, and Theta were involved and the only difference consists in the presence or absence of the chromosome aberration connected with bb^{Df}. The results

TABLE 29a

Spots in experiments involving primarily y, sn^3, bb^{Df}, and θ, without or with a Y chromosome.

EXP.	CONSTITUTION	IND.	SPOTS	% SPOTS	sn^3 1	sn^3 2	sn^3 >2	sn^3 total	y 1	y 2	y >2	y total	$y\ sn^3$ 1	$y\ sn^3$ 2	$y\ sn^3$ >2	$y\ sn^3$ total	y-sn^3 2	y-sn^3 >2	y-sn^3 total	+♂ COL.	OTHER SPOTS‡
1	$\frac{y\ +\ \theta}{y\ sn^3\ bb^{Df}}$ no Y	392	702	180	98	81	203	382	102	30	22	154	11	5	9	25	18	95	113	13	15
1′	$\frac{y\ +\ \theta}{y\ sn^3\ bb^{Df}}$ Y	19	72	379	11	10	21	42	7	4	1	12	1	1	2	4	—	9	9	—	5
2	$\frac{y\ +\ bb^{Df}}{y\ sn^3\ \theta}$ no Y	262	53	20	2	—	—	2	33	8	6	47	—	—	—		—	—		4	—
2′	$\frac{y\ +\ bb^{Df}}{y\ sn^3\ \theta}$ Y	203	167	82	1	2	4	7	60	32	48	140	—	—	—		1	1	2	18	—

‡ Exp. 1. sn^3-$y\ sn^3$ twin spots: 8. y-$y\ sn^3$ twin spots: 4. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 1′. sn^3-$y\ sn^3$ twin spots: 2. y-$y\ sn^3$ twin spots: 2. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 2. y-+♂ col. twin spots: 2.
Exp. 2′. y-+♂ col. twin spots: 4. y-sn^3-+♂ col. triple spot: 1.

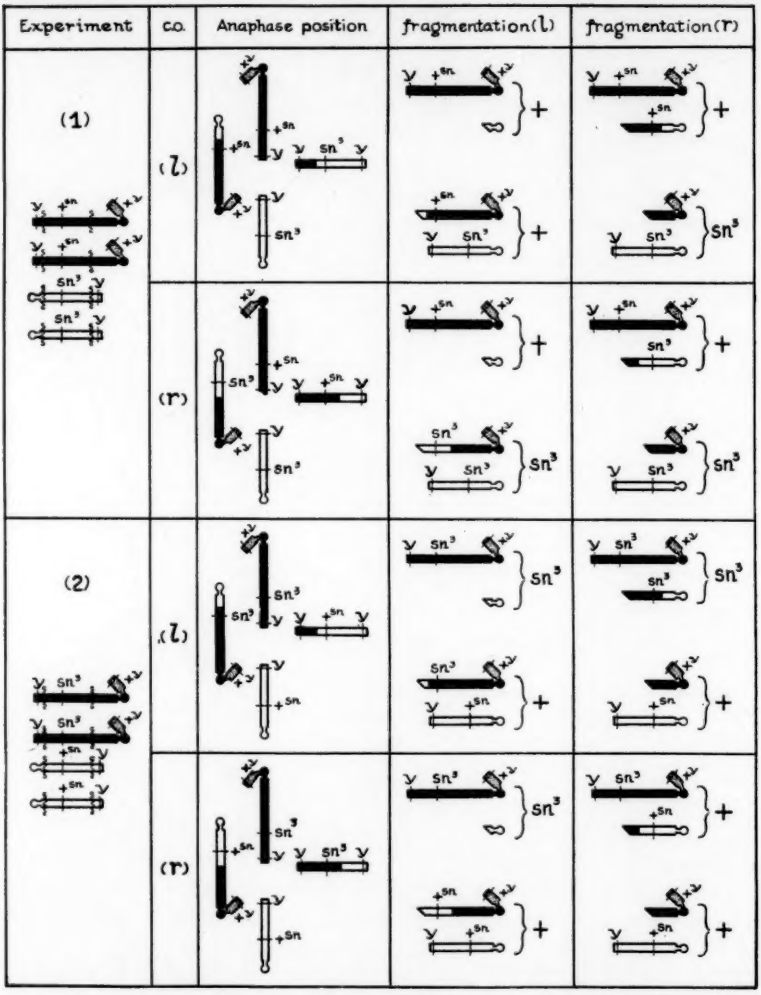


FIGURE 12. Two experiments involving *y*, *sn*^{3}, *bb*^{Df} and Theta (table 29a). The results of x-segregation after crossing over to the left (l) and to the right (r) of *sn*^{3} and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (r) of *sn*^{3}. The lines } indicate the end points of the inverted region.

are fundamentally different. Whereas the numbers of different kinds of spots calculated as percentages of total number of spots in:

Experiment (1) of table 13 were: 80 *sn*^{3}; 13*y*; 3 *y-sn*^{3}; 4 others, the percentages in:

Experiment (1) of table 29a were: 54 *sn*^{3}; 22 *y*; 16 *y*-*sn*^{3}; 8 others.

More striking still is the difference between the experiments 2 without or with *bb*^{Df}:

Experiment (2) table 13: 88 *sn*^{3}; 11*y*; 1 other.

Experiment (2) table 29a: 4 *sn*^{3}; 85 *y*; 11 others.

These differences become intelligible if one takes account of the nature of *bb*^{Df} as representing both a deficiency for the inert region of the X chromosome and an inversion for most of the genetically active region. If we make the same assumptions regarding crossing over within the inverted section, segregation, and fragmentation as those used in the chapter preceding the last we arrive at the expectations presented in figure 12. It is apparent that only *sn*^{3} spots will be produced (not considering at present that some of the + type segregates may lead to areas with male constitution). However, in experiment 1, 45 per cent of the spots were not-*sn*^{3} and it is certain that even a large proportion of the 54 per cent *sn*^{3} spots have to be regarded as ru[illegible]ments of *y* and *sn*^{3} twin spots. In experiment 2 the number of *sn*^{3} spots deviates still more from expectation. Only 2 out of 49 significant spots were *sn*^{3}. Here the extreme rarity of *sn*^{3} spots does not necessarily indicate an equal rareness of crossing over within the inversion. If the most frequent process of crossing over and fragmentation is represented by the process **r,r,** as was the case in certain earlier experiments, then no *sn*^{3} spots will be produced by it in experiment 2, although they would appear in experiment 1 (fig. 12).

As crossing over within the inversion can at most account only for the occurrence of *sn*^{3} spots, other processes have to be looked for to furnish an explanation of the *y* single spots and *y* and *sn*^{3} twin spots. The small number of such spots recorded in table 13 had been shown to be due to somatic crossing over between the Theta-duplication and the homologous region of the right end (mainly) of the X chromosomes. If we consider the same process for the present experiments, we find the following expectations (fig. 13):

(a) Crossing over between Theta and an X chromatid of the chromosome not carrying *bb*^{Df} in experiment 1 leads to twin segregates with the constitutions *y* (3X)-*sn*^{3} (1X, 2 Theta); in experiment 2 to twin segregates *y* (3X)-+(1X, 2 Theta).

(b) Crossing over between Theta and a *bb*^{Df} carrying X chromatid with equational **x** segregation in experiment 1 leads to *y* (3X)-+(1X, 2 Theta) twin segregates and in experiment 2 to *y* (3X)-*sn*^{3} (1X, 2 Theta) twin segregates.

Thus crossing over involving Theta explains the occurrence of *y* spots. Will it also account for the *y*-*sn*^{3} twin spots in experiment 1? It will do so under the assumption that the viability of 1X, 2 Theta segregates is

high enough to give rise to mosaic areas in a large number of cases. This assumption we had found justified during the analysis of experiments involving *y*, *sn*^{3}, *Mn*, and Theta. We can rule out the occurrence of crossing over process **b**, as it leads to *y-sn*^{3} twin spots in experiment (2) which did not occur. This failure in crossing over between Theta and the right end of the bb^{Df} chromosome is in agreement with the probably complete ab-

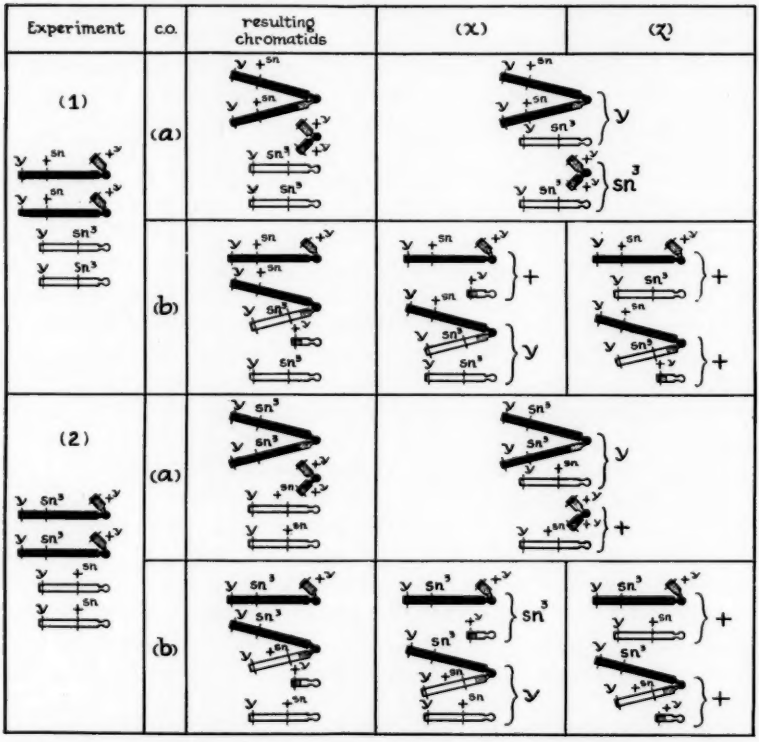


FIGURE 13. Two experiments involving *y*, *sn*^{3}, bb^{Df}, and Theta (table 29a). The results of crossing over between the homologous right regions of Theta and the X chromosomes and of subsequent **x**- and **z**-segregation.

sence of the homologous region in the deficient X chromosome. But if process **a** occurs in experiment 1 it accounts for the *y-sn*^{3} twin spots, the *y* single spots (as rudiments of twin spots), and an unknown proportion of the *sn*^{3} single spots (also as rudiments of twin spots). In experiment 2 it causes the *y* spots. It might be added that crossing over between the left end of Theta and the homologous left end of the X chromosomes yields *y* (2X) spots in both experiments and may contribute to this class.

An independent test of our explanation is provided by a consideration of the sex of spots (table 29b). In experiment 1, 35 *sn*^{3} spots had no male coloration, 7 showed male coloration, and in 34 cases a small spot of male coloration occurred near to the *sn*^{3} bristles. Singed-3 spots both with and without male coloration would be expected from crossing over within the inversion (fig. 12), according to the balance of sex factors brought about by the varying length of the duplicating fragment. In cases where crossing over within the inversion results in a *sn*^{3}, not-male-coloration segregate, the twin segregate will often have a constitution leading to $+^{sn}$, male-color areas. This may account for cases where a spot of male coloration was found near to *sn*^{3}, not-male-colored regions. (It is probable that some

TABLE 29b

Sexual coloration of critical spots from table 29a.

NO. OF EXP. IN TABLE 29a	*sn*^{3}		*y*		*y-sn*^{3}		$\frac{+}{\sigma^{3}}$	$\frac{y-}{+\sigma^{3}}$
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♂ COL.
1	35	7; near 34	13	—	27	4; near 5	13	—
1′	3	—; near 2	1	—	3	—	—	—
2	—	—	6	—	—	—	4	2
2′	1(?)	—; near 1?	13	—	1	—	18	4

In addition exp. 2: 1 *y-sn*^{3}-+ ♀ col. triple spot.

of these cases were *sn*^{3} male-colored single spots in which the *sn*^{3} trichogenic cells have become isolated from the rest of the cells constituting the spot.) Finally, those *sn*^{3} spots which are rudiments of a *y* and *sn*^{3} twin segregate produced after crossing over between Theta and an X chromatid ought to be of male coloration. It is probable that some of the male-colored *sn*^{3} spots owe their origin to the last named process.

All *y* spots in experiment 1 were of "no male color" (13 cases), in agreement with their expected 3X (or sometimes 2X) constitution. A discrepancy, however, exists in the cases of *y-sn*^{3} twin spots. The hypothesis calls for *y* areas with not-male-coloration and *sn*^{3} areas with male constitution. Four such cases were found. Five more cases, in which a small spot of male coloration occurred near the twin areas may in reality be also of the expected *y* ♀, *sn*^{3}♂ kind. But in the majority of critical *y-sn*^{3} spots, (27 cases), no male coloration was visible in either twin area. The existence of this group raises doubts as to the validity of the present explanation. It is possible to make special assumptions to account for the unexpected type but they will not be given as proof is lacking.

The sex of spots in experiment 2 could be recognized in 8 *y* areas only. They were of not-male coloration in agreement with expectation and 2

were accompanied by an area of male coloration as demanded by segregation *y* (3X)-+(1X, 2 Theta).

No close analysis will be attempted for the observed single spots of normal setae with male coloration. Among other possibilities such spots are to be expected from crossing over within the inversion, and in experiment 2 as rudiments of twin spots from crossing over involving Theta.

If, in spite of the discrepancy pointed out, the general explanation given is accepted, then we come to the conclusion that the most frequent type of somatic crossing over is that between the right end of the Theta duplication and the homologous region of the not bb^{Df} bearing X chromatids. In the experiments of table 13 this process is rare but it is understandable that its relative frequency is high when bb^{Df} is present, for the following reasons. Normally the highest frequency of somatic crossing over occurs in the neighborhood of the fibre point. This region is abnormal in a bb^{Df} chromosome lacking most or all of its inert section as well as the *bb* locus and enclosing also one end point of the inversion. These conditions can be expected to interfere with pairing and crossing over of the right ends of the X chromosomes. But they should facilitate homologous pairing of the right end of the normal X chromosome with the homologous region of the Theta duplication.

Apart from the high relative frequency of certain types of spots the absolute frequency of mosaic areas is unusually high in comparison with the similar experiments of table 25a. It may be suggested that the presence of the Theta duplication is responsible for the difference. It contains inert material like a Y chromosome which, as shown above and again below, increases the total frequency of somatic crossing over.

No explanation can be given for the further difference between the frequencies in experiments 1 and 2 of table 29a.

Experiments involving y, sn^3, bb^{Df} and Theta; a Y chromosome present

The ability of a Y chromosome to increase the frequency of spots strongly is again demonstrated by a comparison in table 29a of experiments 1 and 2 with experiment 1′ and 2′. Under the influence of the Y chromosome the frequencies rise from 180 to 379 per cent and from 20 to 83 per cent. No change in relative frequencies of the different types of spots occurred nor did new types of mosaic areas appear. The coloration of the spots in regard to sex (table 29b) showed features similar to those brought out in the preceding section.

Apart from one discrepancy the experiments involving *y*, sn^3, bb^{Df}, and Theta with or without a Y chromosome seem to confirm the explanation proposed for similar experiments in which Theta was not present.

New facts were presented to show that somatic crossing over frequently

TABLE 30a

Spots in experiments involving primarily y, sn^3, Mn, bb^{D_f}, and θ without or with a Y chromosome (see text).

EXP.	CONSTITUTION	IND.	SPOTS	%	sn^3 1	sn^3 2	sn^3 >2	y 1	y 2	y >2	y sn^3 1	y sn^3 2	y sn^3 >2	y-sn^3 2	y-sn^3 >2	y sn^3-sn^3 >2	+ >2	+♂ COL.	+♂ COL.-y 1	+♂ COL.-y 2	+♂ COL.-y >2
1	$\frac{y + + Mnbb^{D_r}}{y\ w^e\ sn^3 + \theta}$	2685	666	25	35	11	97	269	59	188	2	—	—	—	3	—	2	—		—	
						143*			516†			2			3						
2	$\frac{y\ w^e\ sn^3\ Mn\ bb^{D_f}}{y + + + \theta}$	320	207	65	5	3	2	110	33	41	1	—	—	4	6	—	—	2		—	
						10			184			1			10						
3	$\frac{y + Mn\ \theta}{y\ sn^3 + bb^{D_f}}$	28	75	268	11	11	23	4	2	1	3	5	2	4	4	5	—	—		—	
						45			7			10			8						
4a	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb^{D_f}}$ no Y	143	80	56	—	—	—	34	16	18	—	—	—	—	—	—	—	10	1	—	1
									68											2	
b	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb^{D_f}}$ Y	223	502	225	—	—	1(?)	140	86	166	—	—	—	—	—	—	—	71	10	10	18
						1			392											38	
c	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb^{f}}$ Y	151	202	134	—	—	—	63	40	54	—	—	—	—	—	—	—	33	4	2	6
									157											12	

* 51 of these were clearly $+^M$ and 9 *Mn*.
† 71 of these were clearly $+^M$ and 19 *Mn*.

involves the Theta duplication and that the constitution 1X, 2 Theta is viable in hypodermal spots.

Experiments involving y, sn³, Mn, bb^{Df}, and Theta

The conclusions arrived at in the last section are once more found to be in accordance with a series of experiments which contained *Mn* in addition to the genes present in the last group. The results (table 30a) are in marked contrast to those of table 16a, where no *bb^{Df}* chromosome was involved; but they are of the kind to be expected from the theory developed in the last chapter. These experiments were partly made before the role of the Y chromosome in somatic crossing over was known. From independent evidence, however, it is most likely that no Y chromosomes were present in experiments 1 and 2 while an extra Y chromosome probably was present in experiment 4b. In experiments 3 and 4a it is certain that the females inspected had no Y chromosome and experiment 4c was devised in order that a Y chromosome should be present.

TABLE 30b

Sexual coloration of critical spots from Table 30a and from similar experiments.

EXP.	*sn*³		*y*		*y sn*³	*y sn*³ *sn*³	+♂ COL.	*y*-+♂ COL.
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	NO ♂ COL.		
1	1	39‡	—	—	—	—	—	—
2	—	—	23	—	—	—	2	—
3	5	3	—	—	1(?)	1*	—	—
4a	—	—	5	—	—	—	10	2
b	—	—	7	—	—	—	71	38
c	—	—	10	15(?)	—	—	33	12
$++\ Mn\ bb^{Df}$ / $y\ w^e\ +\ \theta$	—	10	—	—	—	—	—	—
$y\ Mn\ \theta$ / $y\ +\ bb^{Df}$ Y	—	—	2	1(?)	—	—	—	4

‡ Determination of sex based on abdominal coloration in 26 spots; on wing length in 2 spots; wing length and sex comb in 1 spot; w^e coloration in 10 spots.

* Determination possible in the *sn*³ spot only.

A short analysis on the basis of our previous findings follows (table 31):

(a) Single crossing over within the inversion and followed by fragmentation of the two-fibre-point chromatid leads to *sn*³ +^{M} and + areas in experiment 1, to + and *sn*³ *Mn* areas in experiment 2, and to *sn*³ *Mn* areas in experiment 3. No mosaic areas are produced in experiment 4.

(b) Crossing over between Theta and the homologous right arm of a not-*bb^{Df}* chromatid leads to the following twin segregates:

Experiment 1: *y*(3X)-*y Mn bb^{Df}* (1X)

Experiment 2: *y*(3X)-*y sn*³ *Mn bb^{Df}* (1X)

Experiment 3: $y(M/M/+;3X)$-sn^3 (1X, 2 Theta)

Experiment 4: $y(M/M/+;3X)$-+(1X, 2 Theta).

The 1X segregates of experiments 1 and 2 are expected to be inviable. Consequently *y* single areas will result in these two experiments. As hypodermal areas with three X chromosomes containing 2 Minute-n and one $+^{Mn}$ allele can be formed according to our earlier findings, *y* and sn^3 twin areas are expected in experiment 3 and *y* areas next to +-(1X)-areas in experiment 4.

TABLE 31

y, sn^3, *Mn*, bb^{Df}, θ

Types of segregates after different kinds of crossovers and fragmentation processes in experiments noted in table 30.

REGION OF C.O. AND FRAGMENTATION	EXP. 1	EXP. 2	EXP. 3	EXP. 4
l,l	sn^3 *Mn*	+ +	dies *Mn*	dies *Mn*
l,r	sn^3 *Mn*	+ sn^3 *Mn*	dies sn^3 *Mn*	dies *Mn*
r,l	sn^3 *Mn*	+ sn^3 *Mn*	dies sn^3 *Mn*	dies *Mn*
r,r	+ *Mn*	+ sn^3 *Mn*	dies sn^3 *Mn*	dies *Mn*
c.o. θ-*X*	$y(3X)$ dies	$y(3X)$ dies	$y(M/M/+)$ $sn^3/\underline{\theta\theta}$	$y(M/M/+)$ $+/\underline{\theta\theta}$

The actual findings lead to the conclusion that the majority of areas are derived from crossing over involving Theta. Although some of the following spots are probably produced by one or the other rarer processes, crossing over involving Theta can account for 516 out of 665 spots in experiment 1, for 184 out of 207 spots in experiment 2, for the *y* and sn^3 twin spots, and, as rudiments of twin spots, for the *y* single and an unknown number of sn^3 single spots in experiment 3, and in experiment 4a for all 80 spots namely the *y* and $+^y$ male-colored twin spots and as rudiments for the *y* single and $+^y$ male coloration single spots. The remaining spots can be derived from different processes of which single crossing over within the inversion accounts for the sn^3 spots in experiments 1 and 2 and for some of them in experiment 3.

In footnote ‡ to table 30a experiment 1 it is stated that of 90 *y* spots on head and thorax which were classifiable as to setae length, 71 were of approximately $+^M$ size and 19 were of M size. Crossing over between Theta and the right arm of the X chromosome leads to *y Mn*/*y*+/*y*+(3X)

spots which show about normal seta size. The *M* type spots therefore must have originated differently, for example, by crossing over between Theta and the homologous left part of the X chromosome. It is not possible to say whether the proportion of the two kinds of *y* spots in this head-thorax sample can or cannot be regarded as representative of the whole sample (see section on somatic segregation and ontogenetic pattern).

The determination of the sex of spots agrees on the whole with the expectation (table 30b). Attention may be directed to the fact that the sex of spots in experiment 1 could be judged not only by the coloration of abdominal spots but also by presence of sex combs, type of eosin eye color, and length of wing.

The absolute frequency of spots was high, even without the presence of a Y chromosome; 25 spots per hundred flies in experiment 1, 65 in experiment 2, 268 in experiment 3 and 56 in experiment 4a. Three of these values are, though somewhat higher, of the same order of magnitude as in experiment 2 of table 29a. The unusually high value of experiment 3 is similar to the one in experiment 1 of table 14. In both cases a $y\ sn^3\ bb^{Df}$ chromosome was present but the cause of the unusually high percentage of spots is not apparent.

The presence of a Y chromosome, in experiments 4b and c raises the total frequency of spots from 56 to 225 and 134 per cent. Besides, in experiment 4c a certain number of unexpected *y* spots with apparently male coloration were found. Some of these most probably belong to the *y* (3X)-+^{y}(1X) twin group; but others were of *y* male coloration indeed. Perhaps segregation similar to that found in the exceptional experiment with $y\ bb^{Df}/sn^3$; Y occurred here.

Information on experiments with $y\ Mn\ bb^{Df}/y\ w^e$ and $y\ Mn\ \theta/y\ bb^{Df}$ Y flies is added in table 30b. The results agree with the rest.

On the whole the theory of somatic crossing over in flies heterozygous for an X chromosome inversion has stood the exacting test provided by the experiments with *y*, sn^3, *Mn*, bb^{Df}, and Theta. If the theory is accepted, the present section adds weight to the deduction that two doses of *Mn* in triplo-X are not lethal to hypodermal areas. This will be of importance for the interpretation of mosaics in flies with a ring-shaped X chromosome (X^c).

Experiments involving the dl-49 Inversion

An X chromosome inversion has been involved also in certain experiments which were discussed earlier. This is the "dl-49" inversion (tables 5 and 13). It is a shorter inversion than the one associated with bb^{Df}, extending only from locus 11± to 42±. Consequently all crossovers from 42± to the right end are outside of the inversion; such crossovers formed

the basis of the discussion. Crossing over within the inverted region leads to sn^3 spots and it is probable that some of the observed sn^3 spots owe their origin to such crossover types.

THE INFLUENCE OF A Y CHROMOSOME ON MOSAIC FORMATION IN FEMALES NOT CARRYING A bb^{D_f} INVERSION

Only one experiment with flies containing two X chromosomes without an inversion gives information on the influence of a Y chromosome under these conditions. It concerns the flies of experiment 2, table 10 which have been discussed before. The female parents of these individuals were XXY so that they themselves consist of XX and XXY flies in about equal numbers. The total frequency of spots in these flies was 72.8 per cent as opposed to 3–12 per cent in experiments 1, 3, and 4 of the same group. The rise in frequency is presumably due to the presence of the Y chromosome in half of the flies. The influence of the Y is of course even greater in this experiment than the number 72.8 per cent indicates, for this represents the average percentage for all individuals of experiment 2, about one-half of which were of XX constitution.

In table 15 it had been shown that 9 out of 11 spots of experiments 1 and 4 of table 10 were not male-colored. However, 17 out of 30 spots in experiment 2 showed male coloration (table 32). According to the simplest

TABLE 32

Sexual coloration of critical spots on y Mn/sn³ flies without or with a Y chromosome (cf. table 10, experiment 2).

TYPE OF SPOT	*y*		*sn³*			*y-sn³*		+♂ COL.
	NO ♂ COL.	♂ COL. NEAR	NO ♂ COL.	♂ COL. NEAR	♂ COL.	NO ♂ COL.	♂ COL.	
Number	2	3	9‡	5†	1*	2	1	7
Y constitution of flies	?	? XXY	XX ? XXY	?	XXY	XXY	?	? ? XXY
Number	2	1 2	1 7 1	1	4	1	2	1 5 2

Totals: not male-colored, **13**; male colored, **17**.

‡ Doubtful: 2 spots.

† Doubtful: 3 spots.

* Doubtful: 1 spot.

form of the theory of somatic crossing over and segregation, no spots with male constitution were expected in these flies. The presence or absence of a Y was tested in 25 cases, the test consisting of a determination of the frequency of non-disjunctional sons and their fertility or sterility. Fertility indicates presence of a Y in the mother, sterility its absence. Lack of exceptional sons does not prove that the mother had no Y chromo-

some but may only be due to too small a total progeny. This test method cannot serve as more than a first survey.

After disregarding the 5 individuals (representing 3 not-male-colored and 2 male-colored spots) which could not be tested there were left 10 not-male-colored and 15 male-colored spots. Only one of the 10 not-male-colored spots could be shown to have occurred on an XXY individual, while 9 out of the 15 male-colored spots were proved to have been on XXY females. The difference between these two distributions is significant ($\chi^2 = 6.25$; P between 0.01 and 0.02). We can conclude that the presence of a Y chromosome increases the number of spots of male coloration in females which do not carry an X chromosome inversion or duplication. The data are not conclusive as to the question whether male-colored spots in such females occur *only* if a Y chromosome is present, since the constitution of the 6 individuals with male-colored spots is not known. They may have been either XXY flies which did not produce exceptions or all or some of them may have been XX. In other experiments at least one case of a male-colored spot was found which occurred in a female in which certainly no Y chromosome was present. This female was supposedly perfectly normal in its chromosomal constitution, but the possible presence of a duplication of new origin cannot be excluded.

The action of the Y chromosome in the experiment just reported reminds us of the numerous male-colored spots in the exceptional experiment with *y bb^{D1}/sn^3*; Y females. There we demonstrated how somatic crossing over in XXY females between an X and the Y may give rise to XX and XXY twin spots, the latter constitution being responsible for the male coloration. It is possible that the occasional male spots found in several of our experiments (table 15, lines 1–3, and others) are due as a rule to the unsuspected presence of a Y chromosome.

If further experiments show that this is true, an at least partial explanation would be available for the fact that BRIDGES (1925) found the sex of spots in his *Mn* individuals to be always male, while most of our experiments with flies of similar constitution yielded mainly female spots. BRIDGES reports that the frequency of spots in his cultures was between 10 and 40 per cent. Considering that BRIDGES paid predominant attention to larger spots, these frequencies are very high. If we assume that supernumerary Y chromosomes were present in BRIDGES' stocks, we have an explanation both for the high frequencies of spots and for their male constitution.

MOSAICS IN FLIES HETEROZYGOUS FOR A RING-SHAPED X CHROMOSOME

Flies heterozygous for a ring-shaped "closed X chromosome" have been reported to have yielded numerous gynandromorphs (L. V. MORGAN,

TABLE 33a

Spots in X^c flies.

Experiments 2–4, 3, 9, 12–14 were inspected with low power only, therefore mainly head-thorax macrochaetae are involved.

EXP.	CONSTITUTION	IND.	SPOTS	sn^3 or f^3			y			$y\ sn^3$			+			y-sn^3 (or f^3)		OTHER SPOTS*
				1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
1	$\frac{y\ X^c}{sn^3}$	153	147	100	12	19	11	2	2							—	1	
2	$\frac{y\ X^c}{sn^3}$	289	25	22	1	1	1	—	—									
3	$\frac{y\ X^c}{y\ sn^3\ bb}$	134	26				1	—	—	23	1	1						
4	$\frac{yX^c}{y\ sn^3\ bb}$ (XX & XXY)	483	59							58	—	1						
5	$\frac{y\ X^c}{w^{bf}f^3}$	—	109	67	9	5	21	—	3							2	1	1
6	$\frac{y\ X^c}{ysn^3\ \theta}$	97	97	71	6	10	5	—	—							2	3	
7	$\frac{y\ X^c}{(w)\ f\ Mn}$	45	47	—	—	1	—	—	3				14	2	26			1
8	$\frac{y\ X^c}{sn^3\ Mn}$	107	151	77	11	5	6	1	2				19	—	26	—	3	1
9	$\frac{y\ X^c}{ywMn\theta}$	27	15										7	—	8			
10	$\frac{y\ sn^3\ X^c}{+}$	—	27	4	—	—	3	—	1	14	1	3						1
11	$\frac{y\ sn^3\ X^c}{w\ bf\ f^3}$‡	110‡	57	11	3	11	2	—	1	17	1	6						5
12	$\frac{y\ sn^3\ X^c}{wmf\ Mn}$	58	35	1	—	—				1	—	3	20	2	6			2
13	$\frac{y\ sn^3\ X^c}{\text{Bld}\ w}$ (Bld-Minute)	—	20				6	—	2	8	2	2						
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	55	45	27	2	14							1	—	1			

‡ In this series most single microchaeta spots were not recorded on account of the uncertainty as to whether they were sn^3 or f^3. The spots listed include mainly macrochaetae spots. Those given in the first column are believed to represent f^3.

* Phenotype of these spots:

Experiment 5: +♂ col.
Experiment 7: +♂ col.
Experiment 8: y-$y\ sn^3$-+♂ col. triple spot (?).
Experiment 10: y (?)-$y\ sn^3$ twin spot.
Experiment 11: $y\ sn^3$-f^3 twin spots: 3-y(?)-$y\ sn^3$ twin spot: 1.-y(?)-$y\ sn^3$-f^3 triple spot: 1.
Experiment 12: $+^y+^M$ (f or $+^f$?).

1926, 1933). At a stage of this investigation when the relation of mosaic occurrence to somatic crossing over had not yet been recognized, varied experiments with this closed X chromosome, which hereafter will be referred to as X^c, were undertaken in order to clear up the problem of origin of mosaic areas. It was found that the frequency of gynandromorphs in flies heterozygous for X^c was not appreciably higher than usual. This had also been true in the later experiments of Mrs. MORGAN and it must be concluded that the original high incidence of gynandromorphs was not due to the closed X condition by itself, but due to either accessory or independent causes which have ceased to exist in the stock.

Although gynandromorphs were practically absent, a high frequency of spots of different kinds was encountered. The data are assembled in table 33a. In order to simplify the presentation, no distinction has been made as to the determination of the seta length in the different groups of spots. In most cases where only microchaetae were involved, no such determination was possible. However, when macrochaetae were included in spots it was seen that they fell into two groups, spots with about normal sized setae and spots with very small setae. A total of 219 spots could be thus classified according to bristle size. Often in cases of sn^3 or f^5 setae no finer distinction as to size was possible. Among the class $+^M$ it was found that generally the size of bristles was smaller than normal although distinctly larger than *Mn*.

In many cases there was a high correlation between the length of setae and the area covered by spots. Thus the total number of small and normal sized bristles in the sn^3 or f^5 group was 67 small and 4 normal among the single-setae spots, while among spots comprising more than one seta all 15 cases which could be classified as to seta size had setae of normal length. Among the *y* spots the relation is: 6 small and 8 normal single-bristle spots and 4 normal sized larger-area spots. Here no correlation is apparent for reasons which will become clear later. Among the *y* sn^3 spots were found: 105 single-seta spots with small and 2 with normal sized setae; larger spots—1 with small, 2 with normal sized setae.

It is immediately apparent that no simple hypothesis of elimination of a chromosome can explain the types of spots found. Such an hypothesis cannot account, among other facts, for the following:

(a) Appearance of numerous $+^M$ spots in experiments 7, 8, 9, and 12. If the *Mn* carrying chromosome were eliminated, *y* or sn^3 spots would be expected, if the not-Minute carrying chromosome were excluded, the remaining constitution would be lethal.

(b) Appearance of *y* and of sn^3 spots in experiments 10 and 11 where the original chromosomes carried both *y* and sn^3 or neither.

(c) The fact that 23 out of 55 critical spots were not of male constitution.

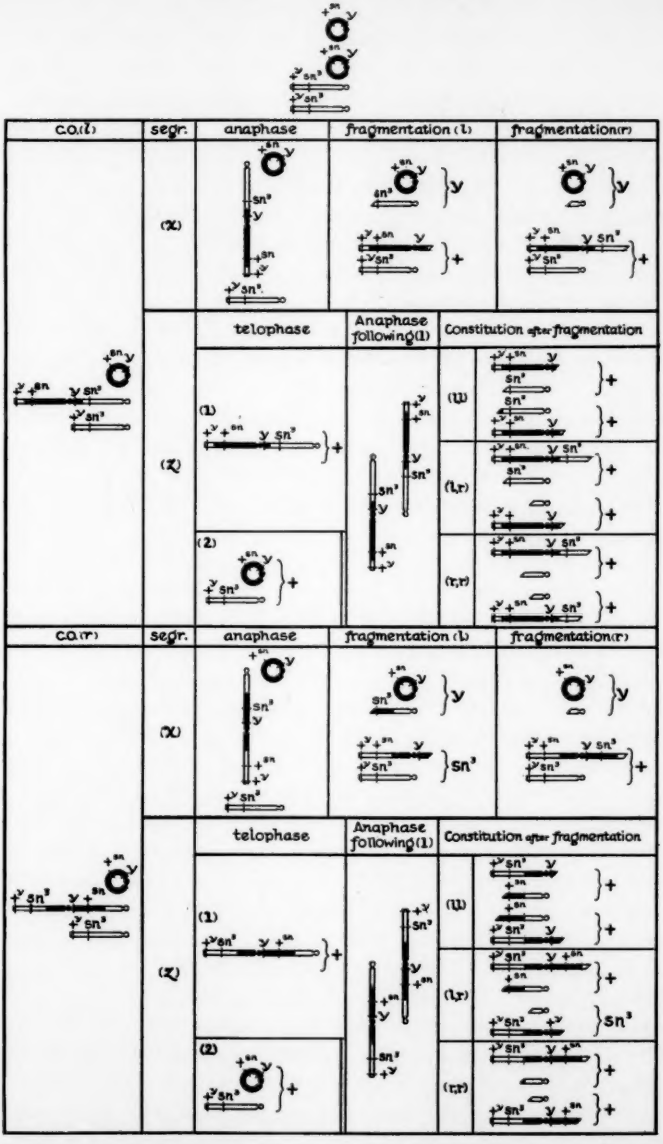


FIGURE 14. *y* X^c/sn^3. The results of crossing over to the left (l) and to the right (r) of sn^3. Subsequent **x**-segregation accompanied by different types of fragmentation of the two-fibre-point chromatid to the left (l) or right (r) of sn^3 or subsequent **z**-segregation followed, in one segregate, by different types of fragmentation (l, l; l, r; r, r) of both two-fibre-point chromatids.

(d) The appearance of abnormally short setae.

The occurrence of different types of spots and the relation between size of setae and amount of area covered by the spot become understandable under the assumption of somatic crossing over and segregation and taking account of the cytological peculiarity of the X^c chromosome, as will be shown now.

In examining the consequences of somatic crossing over we shall restrict ourselves to cases of single crossovers. The analysis will be given here in detail for one case only, represented by the experiments 1 and 2 and concerning flies with the constitution $y\ X^c/sn^3$ (fig. 14). Experiment 5 with $y\ X^c/w^{bf}\ f^5$ flies is very similar.

Crossing over between one strand of the ring chromosome and a strand of the normal X chromosome results in a long, open chromatid consisting of two full X chromatids with two fibre attachment points. This chromatid possesses one sister fibre point of the normal X chromosome on one end and one sister point of the ring chromosome in the middle. A double chromatid of this type has been called a "tandem" by L. V. MORGAN. If separation of sister points proceeds normally, two types of segregation have to be considered:

(1) **x**-segregation. The non-crossover chromatids go to opposite poles, the tandem being subjected to opposite forces on account of its two fibre points tending in different directions. This may result in either **(a)** non-inclusion of the tandem into the daughter nuclei and subsequent elimination, or in **(b)** fragmentation of the tandem between the two fibre points and inclusion of the two fragments within the two daughter nuclei. (A third possibility, the inclusion of the whole tandem chromosome into one daughter nucleus is least probable and will not be discussed.) Process **a** leads to *y* and sn^3 twin segregates with 1X constitution, process **b** to different pairs of segregates depending on the locus of crossing over and of fragmentation: **(l,l), (l,r)** and **(r, r)** to *y* and + twins and **(r,l)** to *y* and sn^3 twins. While the segregates from **a** are both of male constitution, those of **b** contain a deficiency or a duplication for a left section of an X chromosome. Their sex is dependent on the extent of the deficiency or duplication.

(2) **z**-segregation. The non-crossover chromatids go to one pole, the tandem chromatid to the other pole. In this case the two segregates will be of the same genic constitution as the rest of the fly. However, the tandem-containing segregate will divide and its halves undergo fragmentation during the next cell division. The position of the two sister tandem-chromosomes is expected to be of such a nature as would result from sister fibre points going to different poles. Three combinations of fragmentation in the two separate tandems can occur: breaks in both to the

left of sn^3, in both to the right, and in one to the left and one to the right. Unless the two tandems happen to break at identical places unbalanced segregates will result. Their phenotype is in most cases + except in one in which a sn^3 segregate is produced.

When we compare these expectations with the actual results it becomes clear that the process **x** can at best account for only a small number of spots.

(1a) If the tandem were eliminated completely, *y* and sn^3 twin spots with male coloration should appear. Only one twin spot occurred; the proportion of *y* and sn^3 single spots is so unequal that only a few of them can be regarded as vestiges of twin spots. In addition, neither the fact that 11 out of 19 critical spots were not of male constitution nor the small size of most setae in single seta sn^3 spots agrees with the assumption of complete elimination.

(1b) Should the tandem undergo fragmentation during **x**-segregation we would meet a similar lack of agreement between facts and expectation. In three out of the four cases *y* spots will be produced, while *y* and sn^3 segregates result from the fourth. The observed *y* spots are regarded as results of **x**-segregation and fragmentation, but the great majority of spots are single sn^3 spots. Even if one assumes that the unbalanced *y* segregate of the twin cells is inviable, so that only single sn^3 areas are produced, the expectations as to bristle size would be mainly for normal length. For the sn^3 segregate would contain two complete X chromosomes and in addition a duplicating X fragment; such hyperploid condition leads to strong setae (PATTERSON, STONE and BEDICHEK 1935).

(2) The majority of spots can be understood when we assume **z**-segregation to have preceded them. The only segregate resulting in a visibly aberrant area is of the phenotype sn^3. Furthermore it contains only one complete X chromosome besides two fragments which together represent less than a second X chromosome. The sections represented by the two fragments are variable, depending on the locus of fragmentation. Such hypoploid conditions may be expected to result often in short-bristled hypodermal cells of low viability. The 122 sn^3 single-seta spots of experiments 1 and 2 and the 67 f^5 single-seta spots of experiment 5 correspond to this type.

To account for the smaller number of sn^3 spots which are of larger size and possess bristles of normal length one can assume both that some of them represent special hypoploid conditions due to **z**-segregation which are favorable in respect to genic balance and also that they are results of double crossing over to both sides of sn^3, a process which will lead to normal segregation of two and two X chromosomes and homozygosity for sn^3 in one segregate.

The sexual coloration of critical spots in experiments 1 and 5 was male in 9, not-male in 10 *sn*^{3} spots and as regards *y* spots male in 1 and not-male in 2 cases (table 33b). No very definite expectation on the basis of the proposed theory is possible, since hypoploid conditions may lead to both types of sexual characteristics (which involve intersexual conditions) according to the length and region of the duplicating fragments involved. Those *sn*^{3} spots which are derived from double crossovers are expected to be of normal female constitution.

TABLE 33b

Sexual coloration of critical spots from table 33a.

	sn^2 OR *f*^{3}		*y*		*y sn*^{3}		+♂^{3}	*y-sn*^{3}	*y sn*^{2}-*f*^{3}	*y sn*^{2}-*y*(?)
	NO ♂^{3}	♂^{3}	NO ♂^{3}	♂^{3}	NO ♂^{3}	♂^{3}		♂^{3}	♂^{3}	NO ♂^{3}
1	9	8	2	—						
5	1	1	—	1			1			
6	3	4						1†		
7				1*			1			
8	4	1						1		
10					1	—				
11		6				1			2	1
14	1	2								
	18	22	2	2	1	1	2	2	2	1

Totals: not male colored, **22**; male colored **31.**

† Determination of sexual coloration only possible in *sn*^{3} area.

* Sex comb.

If one applies the theory of somatic crossing over, segregation, and fragmentation of the tandem chromosome to the other experiments reported in table 33, one arrives at specific expectations in each case with regard to the major types of spots. The derivations have to take account of the different regions of the chromosomes in which crossing over and fragmentation can occur and are rather lengthy in some cases. The resulting expectations are summarized in table 34 together with the observed facts. There is a good agreement both as to kind of main spots and as to the larger frequency of spots produced in consequence of **z**-segregation as opposed to **x**-segregation. One exception with respect to the latter point is found: In experiment 10 the number of *y sn*^{3} spots exceeds that of *sn*^{3} spots, although the former are expected from **x**- and the latter from **z**-segregation. Such a case probably would lose its peculiar character if one could adjust the expectations to the supposedly different frequencies of crossing over in different regions. But no attempt toward a finer analysis of the data presented will be made here. Such an analysis should include addi-

tional facts to be derived from the use of the newly discovered closed X chromosome which carries the normal allele of yellow.

TABLE 34

Expected and observed spots in experiments with X^c flies.

EXP.	CONSTITUTION	EXPECTATION‡		OBSERVED SPOTS	
		(z)	(x)	LARGEST CLASS	2ND LARGEST* CLASS
1, 2, 5	$\frac{y\ X^c}{sn^3 \text{ or } f^5}$	sn^3	y, y-sn^3	sn^3	y
3, 4	$\frac{y\ X^c}{y\ sn^3\ bb}$ (Y)	$y\ sn^3$	$y\ sn^3$	$y\ sn^3$	—
6	$\frac{y\ X^c}{y\ sn^3\ \theta}$	sn^3	sn^3	sn^3	—
7, 8	$\frac{y\ X^c}{sn^3\ Mn \text{ or } w\ f\ Mn}$	$+^M$, sn^3	y, y-sn^3	$+^M$	—
9	$\frac{y\ X^c}{y\ w\ Mn\ \theta}$	$+^M$	$+^M$	$+^M$	—
10	$\frac{y\ sn^3\ X^c}{+}$	sn^3	$y\ sn^3$, y	$y\ sn^3$	sn^3, y
11	$\frac{y\ sn^3\ X^c}{w^{bf}\ f^5}$	f^5, sn^3	$y\ sn^3$, y, $y\ sn^3$-f^5 etc.	f^5	$y\ sn^3$
12	$\frac{y\ sn^3\ X^c}{w\ m\ f\ Mn}$	$+^M$, sn^3	$y\ sn^3$, y	$+^M$	—
13	$\frac{y\ sn^3\ X^c}{\text{Bld } w}$ (Bld-Minute)	y, $y\ sn^3$	$y\ sn^3$, y	$y\ sn^3$	y
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	$+^M$, sn^3	$+^M$, sn^3, y-sn^3	sn^3	—

‡ Expectation in case of y, sn or f not specified in respect to M or $+^M$.
* Only given if larger than 10% of largest class.

There was an opportunity, in experiment 4, to discover a possible influence of the Y chromosome on the occurrence of spots. Part of these flies carried a Y chromosome, but no effect became apparent.

In addition to the demonstration that the theory of somatic crossing over can give an explanation for spots in flies with a closed X chromosome a new result is contained in these experiments. This is the relative frequency of **x**- and **z**-segregation. While in the experiments discussed in

former sections of this paper **z**-segregation does not lead to mosaic areas and its frequency therefore cannot be directly determined, in the present cases it occurs far more often than **x**-segregation. It appears probable that this is a consequence of particular conditions brought about by the presence of the tandem chromosome. These seem to result in preferential chromatid segregation, so that the two non-crossover strands go most frequently together to one pole and the tandem with its two fibre points to the other pole.

Changes in ring-shaped chromosomes during somatic divisions have been demonstrated cytologically by McCLINTOCK (1932) in maize. She has pointed out that somatic crossing over is probably responsible for these changes and that they are correlated with the origin of mosaics.

SEX-LINKED MOSAIC AREAS IN SUPERFEMALES AND IN MALES

No special study of sex-linked spots in flies of not-female constitution was made but some incidental observations seem worth recording.

Superfemales (3X+2A)

(1) *The three X chromosomes free.* Of 11 superfemales of the constitution *y sl*^{2} *bb*^{D1}/*y sn*^{3}*bb*/*sn*^{3} inspected, 3 were free from spots, while the remaining flies exhibited 18 *y* spots (1 seta: 12 spots; 2 setae: 3 spots; >2 setae: 3 spots). In five other flies of the same constitution 5 *y* spots and 1 *y* and *sn*^{3} twin spot (2 setae) were found.

(2) *Two attached X chromosomes, one free*: 14 $\widehat{yy}$/*sn*^{3}*bb* individuals possessed 3 *y* spots (1 seta: 2 spots; 2 setae (no ♂ col.): 1 spot); 19 $\widehat{yy}$/*y sn*^{3}*bb*^{D1}(Y?) individuals possessed 4 *y sn*^{3} spots (1 seta: 1; 2 setae: 10; >2 setae: 2).

The interpretation of these spots according to the theory of somatic crossing over is obvious.

Males

Spots involving segregation of sex-linked genes generally cannot be discovered in males. The only possibility which should give visible mosaic areas would be a segregation of two sister X chromosomes into one nucleus and resulting in a female spot. No case of this kind was encountered but the available data are not extensive enough to exclude the occurrence of such spots.

The situation is different in case of presence of an X chromosome duplication. Males which, besides a *y sl*^{2} *bb*^{D1} X chromosome and (most probably) a Y chromosome, possess a separate chromosome consisting of a Theta duplication attached to the short arm of a Y chromosome have not-yellow body color and setae. They show *y* spots with *y* setae very frequently. This would be understandable if somatic crossing over between

homologous parts of the Theta chromosome and the Y chromosome and normal segregation occurs.

It should be added that 65 males of the constitution "*y sn*^{3}; Theta, Y^s; no free Y" did not exhibit spots; nor did 135 "*y*, Theta; Y" and 85 "*y sn*^{3}, Theta; Y" males.

RELATIONS BETWEEN SOMATIC CROSSING OVER AND THE ONTOGENETIC PATTERN

The frequency of somatic crossing over is dependent on different factors. An influence of the environment was shown by BRIDGES (MORGAN, STURTEVANT and BRIDGES 1929) who found a decrease in number of spots with the progressing age of the culture, independent of the age of the mother. Experiments in our laboratory demonstrate an effect of varying temperature (STERN and RENTSCHLER 1936). Genetic factors which influence the percentage of spots are the Minutes, an extra Y chromosome, and probably different other factors, as judged from the variability of our results.

The dependence of the frequency of spots on different agents results in the appearance of different types of mosaicism. We thus have a parallel to the variable frequencies of piebald areas in mammals or to mosaic conditions in many organisms in general. It is possible to carry the comparison further. It can be shown that not only the frequency but the size and distribution of spots over the body is variable and dependent on different agents. This means that the time and frequency of the origin of segregates is independently variable in different regions of the developing organism. That this is true for mosaics which have been ascribed to mutations of unstable genes is well known (DEMEREC 1935). The following gives a corresponding account for spots which are known to be due to somatic crossing over.

With regard to differences *in time* of occurrence we shall refer only to two earlier statements. It was found (1) that the proportion of "1-seta spots" to "larger than 1-seta spots" is different in different experiments (table 3; see also other tables) and (2) that left crossovers are represented by large and small spots in some experiments (table 8) while they result only in single-seta spots in others (tables 10, 12). The causes for the variability in time of origin of spots are not known for the experiments referred to under (1). As to (2), it seems that the presence of *Mn* is correlated with the shift in occurrence of double crossovers.

The dependence of incidence of somatic crossing over on the spatial ontogenetic pattern, that is, on the conditions offered by different body regions of the larvae will be illustrated by a series of results. Such a dependence first became evident from the analysis of table 3, where the

TABLE 35

Distribution, in percentage of total, of spots over head, thorax, and abdomen in different experiments.

CONSTITUTION	NO. OF SPOTS	HEAD	THORAX	ABDOMEN
sn^3 Mn/y g^2 ty	120	5	58	37
y/sn^3	212	4	43	53
y Mn bb^{Df}/y w^e sn^3 θ	666	4	36	60
y w^e sn^3 Mn θ/y bb^{Df}	80	1	3	96
y w^e sn^3 Mn θ/y bb^{Df} Y	203	0	1	99
Setae Inspected*	—	4	30	66

* In this and in the following two tables the term "setae inspected" refers to the percentage of all inspected setae which were located on the respective body regions. For detailed account see "Methods" and table 1.

average increase of number of spots in Minute flies as compared with not-Minute flies was lower for the abdominal regions than for the head and thorax or from the fact that the proportion of single-seta spots to larger spots was lower in the abdomen than in the head-thorax region (table 3). Other examples of greatly varying distribution of spots over the three main body regions are given in table 35. While this table is concerned with total number of spots, a more detailed analysis can be derived from a separate consideration of different types of spots (table 36). In experiment 1 no different distribution of sn^3 single and y and sn^3 twin spots is expected, because nearly all sn^3 single spots are regarded to be rudiments of twin spots. This expectation is fulfilled, as the last columns indicate. With re-

TABLE 36

Distribution, in percentage, of different types of spots over head, thorax, and abdomen.

CONSTITUTION	SPOTS		HEAD	THORAX	ABDOMEN	χ^2	P
	TYPE	NO.					
(1) y/sn^3	y	59	10	48	42	y, twin: 9.614	<0.01
	sn^3	82	2	35	63	y, sn^3: 8.186	0.01–0.02
	y-sn^3 twin	67	0	40	60	sn^3, twin: 2.101	0.03–0.05
(2) y bb^{Df}/sn^3	y	109	0	16	84	y, sn^3: 2.203	0.01–0.02
	sn^3	60	0	8	92		
(3) y sn^3 $bb^{Df}/y\theta$	y	151	0	8	92	y, sn^3: 23.321	<0.01
	sn^3	382	0	1	99	y, twin: 6.885	<0.01
	y-sn^3 twin	113	0	1	99	sn^3, twin: 0.020	> .99
(4) y Mn bb^{Df}/y w^e sn^3 θ	y	497	2	33	65	y, sn^3: 14.614	<0.01
	sn^3	134	8	34	57		
Setae Inspected		—	4	30	66		

spect to the distributions of *y* single and *y sn*^{3} twin spots as well as for that of *y* and of *sn*^{3} single spots, the χ^2 test points to significant differences. The majority of *y* spots represent rudiments of twin spots and should be distributed in the same way as the *sn*^{3} single and the twin spots. Therefore the deviation is interpreted to mean that those *y* spots which owe their origin to crossing over between *y* and *sn*^{3} have a distribution which is distinguished from the rest. The data indicate that the head and thorax regions are more favorable to left crossovers than to crossovers near the fibre point.

TABLE 37

Distribution, in percentage, of different spots over the abdominal tergites 1–7.

CONSTITUTION	SPOTS		ABDOMINAL TERGITES						x	P
	TYPE	NO.	1+2	3	4	5	6	7		
y/sn^3	*y*	24	4	25	21	29	21	0	*y*, *sn*^{3}: 1.403	0.8– 0.9
	sn^3	51	4	22	33	24	17	0	*y*, twin: 8.787	0.05–0.1
	y-sn^3 twin	40	10	25	33	17	15	0	*sn*^{3}, twin: 1.861	0.7 –0.8
$y\ bb^{D_f}/sn^3$	*y*	55	5	13	32	41	9	0	*y*, *sn*^{3}: 16.390	<0.01
	sn^3	91	2	40	29	27	2	0		
$y\ sn^3\ bb^{D_f}/y\ \theta$	*y*	139	19	18	22	30	11	0	*y*, *sn*^{3}: 20.590	<0.01
	sn^3	376	4	21	20	29	25	1	*y*, twin: 13.277	<0.01
	y-sn^3 twin	112	4	22	20	32	22	0	*sn*^{3}, twin: 0.8417	0.5–0.7
$y\ Mn\ bb^{D_f}/y\ w^a\ sn^3\ \theta$	*y*	323	13	30	32	21	4	0	*y*, *sn*^{3}: 35.0392	<0.01
	sn^3	77	5	23	21	30	20	1		
Setae Inspected		—	10	13	17	24	24	12		

No significantly different distribution of the two types of spots is apparent in experiment 2. Both types result mainly from single crossovers within the inversion. An analysis is impeded by the possibility that different body regions may conceivably be variable in their effect on the viability of hypo- and hyperploid cells and thus lead to a differential survival of spots. This factor may play a role also in the other experiments to be discussed.

In experiment 3 no significant deviation of the distribution of *sn*^{3} single and twin spots occurred, although these spots owe their origin largely to different processes. However, the distribution of *y* spots differs from both that of *sn*^{3} and that of twin spots. While the first deviation is not surprising, since the two kinds of spots are mainly results of different crossovers, the distribution of *y* single and of twin spots should be identical, for the *y* spots are regarded to be rudiments of twin segregates. Possibly viability differences of the *y* and *sn*^{3} twin segregates in different body regions are responible for the result.

In experiment 4 the distributions again differ significantly. Dependence of the occurrence of the two kinds of crossovers or of the viability of segregates upon conditions in different body regions seem to be involved.

The same four experiments which were considered with regard to head-thorax-abdomen distribution of spots have been analyzed as to location of spots on the tergites of different abdominal segments (table 37). In experiment 1 no significant deviation in distribution occurred between *y* and *sn*^{3} single spots nor between *sn*^{3} single and twin spots, while the *y* single and the twin spots were probably significantly different. Taking

TABLE 38a, b

Distribution of y and sn^3 *spots over different body regions. Only spots from yMnbb*^{Df}*/yw sn*^{3} *θ flies of tables 36, 37 are included which occurred on individuals bearing at least one of each kind of spot.*

38a

SPOTS		HEAD	THORAX	ABDOMEN
TYPE	NO.			
y	29	2	6	21
sn^3	37	2	16	19

38b

	ABDOMINAL TERGITES				
NO.	1+2	3	4	5	6
21	4	7	4	4	2
19	1	5	3	6	4

account of the smaller numbers, the earlier comments on this experiment seem to apply here likewise. In experiment 2 the *y* and *sn*^{3} spots are doubtless distributed differently on the abdomen. In experiment 3 the similarities and differences resemble those of the distributions over head-thorax and abdomen. Lastly, in experiment 4, a striking dependence of type of spot on the abdominal region is apparent, showing a peak in frequency for *y* spots on the third and fourth segments and for the *sn*^{3} spots on the fifth segment.

It might be asked whether the differential distribution of different kinds of spots may be due to their occurrence on different individuals. To answer this question tables 38a and b are presented, in which are included all those *y* and *sn*^{3} spots of experiment 4 which occurred on individuals bearing at least one of each type. The numbers are too small to give significantly different distributions. However, in the abdominal series it is evident that the trend of frequencies for the *y* and *sn*^{3} spots coincides well with those of table 35, experiment 4.

The interpretation of the observed differences in abdominal distribution has to take account again of both differences in incidence of crossovers and of viability values. But it seems improbable that the survival values of different segregates vary enough in different tergites to cause the observed patterns by themselves. The participation of the body pattern as differ-

ential in regard to occurrence of somatic crossover types is regarded to be a contributing if not the main factor.

A comparison of the observed distribution of frequencies of spots with the numbers of setae inspected in different body regions (see last line of tables 35–37) shows that no random distribution took place. This is a further demonstration of the influence of body pattern on crossover occurrence. A finer analysis will depend on detailed knowledge and comparison of the developmental events within the different imaginal discs.

DISCUSSION

The evidence presented in this paper shows that mosaic areas on the body of *Drosophila melanogaster* appear in a varying percentage of flies whenever they are heterozygous for genes whose homozygous effect is recognizable in small spots. Theories as to the causation of these spots have to be based on somewhat intricate deductions. The observable phenomenon is limited mainly to yellow or singed single spots, or yellow and singed twin spots, in different proportions and with a few other attributes such as sexual coloration or seta length. The test of any theory as to the mechanism of spot production has to consist in its application to a varied group of genic combinations. Such a test is able to exclude definitely theories which cannot account for the actual facts. But if it succeeds in giving a satisfactory basis for them, it cannot claim a final "proof." In our special case we can say that the theory of chromosome elimination has been shown definitely unsatisfactory. The validity of the theory of somatic crossing over and segregation rests on its faculty to explain the manifold results presented in this paper. Throughout the text the word "assumption" has been used freely in order to leave no doubt as to the procedure of deduction. But it should be noted that most of these assumptions are justified from other experience.

There is a higher degree of safety in the discussion of somatic crossing over in cases where the X chromosomes were normal than in those where inversions or the closed X chromosome were involved. In these latter cases the claim is made that the proposed theory is able to account for most facts (some notable discrepancies have been pointed out), but the possibility of inventing other schemes should be stressed.

This analysis of somatic crossing over can by no means be regarded as complete. It was restricted in most cases to a consideration of single crossovers, although the rarer occurrence of double crossovers has been ascertained. No attempt was made to discuss multiple crossing over in detail, as for instance the relation of the different crossovers to different chromatids, or questions of interference. Neither has the probability been dealt with that somatic crossover processes may occasionally occur consecu-

tively for two or more cell generations. Such repeated crossovers are thought responsible for the occasional triple spots as well as for certain twin spots. A filling out of these gaps in our knowledge although desirable is perhaps not likely to lead to results of general significance. But this might be different with respect to other problems raised in the course of study. To name some of these: what is the "physiological Minute condition" which is responsible for somatic crossing over? Why is there correlation between the locus of Minutes and the region of crossing over induction, between sex-linked Minutes and crossing over in the X chromosome, between autosomal Minutes and crossing over in the autosome, between locus of Minute to the left or to the right of the fibre point and crossing over in the corresponding arm? What is the mechanism of the Y chromosome effect on crossing over? What is the explanation for the exceptional, but self-consistent experiment with $y\ bb^{D1}/sn^3$; Y females?

The mechanism of mosaic formation rests *primarily* on the occurrence of somatic crossing over. If the mechanism of fibre point separation in mitosis remains undisturbed, as has been shown to be true, then genic segregation is a *necessary consequence* of crossing over. There is thus no need to assume two separate processes (1) somatic crossing over and (2) somatic segregation.

Abnormalities in chromosome behavior as causes of mosaic formation have been recognized before. Elimination of chromosomes or non-disjunction has been the most general explanation, for example, in gynandromorphs of Drosophila (MORGAN and BRIDGES 1919), or in certain types of endosperm mosaics in corn (EMERSON 1921, 1924). The idea of possible somatic crossing over was introduced by SEREBROVSKY (1925) in an interesting paper on the appearance of aberrant feathers on chickens of different genetic constitutions. SEREBROVSKY sums up his findings by stating "crossing over cannot be either proved, or denied" and he did not recognize it as the *cause* of somatic segregation. A different interpretation of his cases was consequently given by P. HERTWIG and RITTERSHAUS (1929) and by the present author (1928a, 1933). But it must be said now that SEREBROVSKY probably came nearer to the right explanation than his critics, although certain difficulties remain.

Actual segregation of the chromosomes among somatic cells was genetically demonstrated first by PATTERSON (1929a, b; see also FRIESEN 1935) who subjected Drosophila larvae to X-rays and obtained twin spots, besides many single spots. The author "cannot state definitely the nature of the mechanism which produces the segregation. It almost certainly involves some form of synapsis . . . in the somatic cells." On the basis of our present knowledge we believe the mechanism to have been somatic crossing over as suggested earlier by PAINTER (1934). And we are

inclined to regard most cases of mosaic formation in X-rayed larvae not as results of elimination of parts of chromosomes as assumed before (PATTERSON 1930) but as indication that X-rays can induce crossing over in somatic cells.

There are some cytological findings which bear on this discussion. MCCLINTOCK (1932) obtained cytological evidence of different chromosome constitution in diverse cells of a variegated corn plant. Her finding of different degrees of increase or decrease in length of a ring-shaped chromosome in different cells pointed strongly to some kind of somatic crossing over.

In *Drosophila melanogaster* KAUFMANN (1934) could show the occasional presence of chiasmatype-like configurations between homologous chromosomes in somatic cells, before genetic evidence for somatic crossing over was available. And PETO has recently shown (1935) that chiasmata are formed in cells of root tips of *Hordeum vulgare* under the influence of radiation, a cytological correlate to PATTERSON'S experiments.

How frequently mosaic production is to be regarded as caused by somatic crossing over can be determined by new experiments only. In *Drosophila melanogaster* it seems by far the most prevalent mechanism. In all our experiments in which products of elimination or non-disjunction could be distinguished from those of somatic crossing over and segregation the majority of spots was understandable only by assuming the latter process, whereas the small minority could be explained on either hypothesis. This was true both for the smaller spots and for the occasional larger spots comprising more than one imaginal disk. There is one exception to this statement: the gynandromorphs described by MORGAN and BRIDGES (1919) and other workers cannot be explained by crossing over and segregation. This is perhaps significant. The somatic pairing of homologous chromosomes which is typical in Diptera becomes visible first in the prophase of the second cleavage division (HUETTNER 1924). As most gynandromorphic conditions originate during the first division a causal connection becomes probable.

One might suspect that the somatic chromosome pairing in Drosophila would facilitate the occurrence of crossing over. This would point to crossing over as a cause of mosaic formation in Diptera mainly. But as we do not know the reason why certain cells do undergo crossing over we are hardly justified at present in drawing this conclusion. On the other hand we should hold open the possibility of non-homologous somatic crossing over as it seems to occur in corn (MCCLINTOCK 1932, JONES 1936). In Drosophila no evidence for non-homologous crossing over is available.

Many cases of inherited mosaic formation have been described as consequences of mutations of "unstable" genes (DEMEREC 1935). The present

writer (1935) has proposed a hypothesis according to which the behavior of unstable loci should be regarded not as an internal change in a gene but as a result of "mechanical" changes at the locus, brought about by somatic crossing over. A model for such unstable loci was elaborated. Recently SCHULTZ (1936) has proposed a different cytological configuration in cases of unstable loci, which, being based on direct observation, is superior to the original speculation although probably not final either. An essential part of SCHULTZ'S scheme is again the assumption that somatic crossing over changes the cytological configuration and thus leads to mosaics.

The finding of somatic crossing over throws some light on the mechanism of chromosome separation in mitosis. Normally the passing to different poles of chromosome halves implies a separation of whole sister strands. In cases of crossing over separation may occur in the normal way for one particular point only while the rest of two sister chromosomes may pass to the same pole. This particular point is the fibre attachment locus. We have here a genetic demonstration of its role in mitosis.

That the occurrence of crossing over without reduction of chromosomes is of significance for theories of meiosis and mitosis needs hardly to be pointed out.

The value of somatic segregation as a tool for the analysis of gene action is obvious. The process has been successfully used by DEMEREC (1934 and later) in his studies of the action of small deficiencies in hypodermal segregates (cf. also STERN 1935). A few more facts have come to light in the foregoing pages: viability of hypodermal areas containing one X chromosome and two Theta duplications; or one X chromosome and one long X duplication of varying length; or two X chromosomes and similar duplications; or three X chromosomes containing two Minute-n loci. All these constitutions are lethal to zygotes. The genic unbalance represented by them is thus not able to sustain full ontogenetic development but is compatible with division and differentiation of cells of imaginal discs.

SUMMARY

(1) Mosaic areas on the body of *Drosophila melanogaster* appear on flies which are heterozygous for genes whose homozygous effect can be recognized in a small spot.

(2) The frequency of spots is increased by the presence of Minute factors.

(3) Spots of sex-linked characters occur with higher frequency when either sex-linked or autosomal Minutes are present, but sex-linked Minutes are more powerful than autosomal ones. Autosomal spots are more frequent in the case of presence of autosomal Minutes than of sex-linked

Minutes. Different Minutes show different degrees of ability to induce spot formation.

(4) The mechanism of mosaic formation is not based on simple elimination of chromosomes but on processes of somatic crossing over involving two strands of a four strand group. Segregation of the four strands occurs in an equational, typically mitotic mode in respect to the fibre points. It leads to homozygosis of originally heterozygous genes. No reduction of number of chromatids takes place in normal cases. These conclusions are derived from an analysis of types and frequencies of twin and single spots and of the number of X chromosomes present as judged by the secondary sexual characters of favorable spots. Interpretations based on experiments with certain combinations of genes have been verified by tests of validity in other combinations of the same genes.

(5) The increase of frequency of sex-linked spots is not directly dependent on the localized, material constitution of the chromosomes involved in somatic crossing over but rather on the general "phenotypic Minute reaction" in development.

(6) The relative frequencies of somatic crossovers in different regions of the X chromosomes are different from those of germinal crossovers. Somatic crossing over is more frequent near the fibre point. The presence of Minute-n accentuates this shift.

(7) The X chromosome duplication "Theta" frequently undergoes somatic crossing over with the X chromosome—more frequently in the homologous right than in the homologous left regions. Germinal crossing over involving Theta is very rare.

(8) Somatic crossing over involving Theta followed by equational segregation leads to twin segregates of the constitution 3X chromosomes-1X chromosome.

(9) The apparently exceptional behavior of the bobbed character, which does not become visible in spots, is understandable under the assumption that no somatic crossing over occurs to the right of the bobbed locus.

(10) Somatic crossing over involving the sex chromosome occurs in superfemales and in males.

(11) Somatic autosomal crossing over takes place in both sexes, though more frequently in females. A peculiar specificity of the Minute effect leads to crossovers in that arm of the third chromosome in which the Minute itself is located. Most crossovers are concentrated near the fibre point region.

(12) Somatic crossing over between X chromosomes heterozygous for the bb^{D1} inversion occurs within the inversion. It leads to a chromatid

which possesses no fibre point and is thus eliminated, and to a complementary chromatid with two fiber points. This chromatid becomes fragmented and each fragment is included in a daughter nucleus.

(13) When Theta is present in cells heterozygous for the bb^{Df} inversion the most frequent type of somatic crossover involves Theta and the not-inverted, not-bb^{Df} chromosome. (A discrepancy is pointed out between this interpretation and the observed facts.)

(14) The presence of an extra Y chromosome in flies discussed under (12) increases the frequency of somatic crossing over within the inversion to the right of sn^3 as well as the frequency of fragmentation of the two-fibre-point chromatid, also to the right of sn^3.

(15) In flies discussed under (13) the presence of an extra Y chromosome increases the frequency of crossovers involving Theta.

(16) An exceptional series of cultures with XXY females gave results which can be interpreted as caused by somatic crossing over between X and Y chromosomes, leading to XXX and X segregates.

(17) Somatic crossing over in flies heterozygous for a ring-shaped X chromosome leads to a two-fibre-point "tandem" chromatid. Segregation occurs preferentially so that the two non-crossover chromatids go to one pole and the tandem chromatid to the other. In the following division the tandem chromatid becomes fragmented.

(18) In different experiments certain segregated constitutions are not sufficiently viable to give rise to mosaic areas. Others, though not viable as zygotic constitutions, permit the formation of hypodermal spots.

(19) Under different genetic conditions different patterns of mosaics are formed. The proportion of small to larger spots can vary. In Minute-n flies crossovers to the left of *Mn* occur later in development than crossovers to the right. Various genetic constitutions have differential effects on frequency and size of spots in various body regions. Different types of spots in flies of the same constitution are differently distributed over the head, thorax and abdomen or over the different abdominal segments.

(20) In the discussion, a short survey is given with reference to mosaic formation in general and its relation to somatic segregation.

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GENES FROM RADIUM TREATMENT AFFECTING POLLEN-TUBE GROWTH IN DATURA[1]

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INTRODUCTION

GENES affecting pollen-tube growth in *Datura stramonium* come under two general categories: (1) those which may be transmitted through both the eggs and the pollen, with recognizable abnormalities in the growth of the pollen tubes, and (2) those which are not transmitted through the pollen, but are transmitted maternally. The first category is illustrated by the gene for tricarpel (*tc*) (BUCHHOLZ and BLAKESLEE 1927). Plants homozygous for tricarpel are carried in our stock cultures and maintain themselves as pure lines, while types of the second category must be carried as heterozygotes.

The present paper has to do with the second category of pollen-tube genes. They comprise the genes giving pollen-tube growth distributions of the types V, VIa, VII, and VIIb, which were briefly described in a recent account (BUCHHOLZ and BLAKESLEE 1932a). In an earlier description of some of these gene types (BUCHHOLZ and BLAKESLEE 1930) we called them "lethals of pollen-tube growth," although they might better be spoken of as pollen-tube genes. It should be remembered that a gene of this kind is lethal only to the male gametophyte which carries it. In consequence, due to segregation in the reduction divisions, half of the pollen tubes from a heterozygous parent show the character and half are normal. These types may be recognized through growth tests of the pollen of the plants which are heterozygous for them, the so-called carrier plants.

Nothing may be found in the external morphology of the carrier plants which would distinguish them from non-carriers. The only direct tests which positively distinguish carriers from non-carriers are tests of the pollen when grown on the stigma and in the style of a test flower. For many of these genes any flower of a Datura may serve as a test flower,

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though the pistils of standard Line 1 or self pollinations are used in our routine work (BUCHHOLZ 1931 and BUCHHOLZ and BLAKESLEE 1932a).

From among more than 40 genes affecting pollen-tube growth, which had been recognized up to 1931 and tested for their egg-transmission, we selected for special study five which belong to the second category. We have symbolized these: *s-1*, *s-2*, *sb-1*, *sb-2*, and *lp*. These carrier strains may be continued by identifying and selfing a carrier plant in each generation. Our practice, however, is to keep these genes in our standard Line 1 race by each generation pollinating some of the carriers with the pollen of Line 1. These genes are now in the 4th to 6th generation of backcrossing to Line 1 and, counting two selfed generations, they are from five to seven generations removed from the carrier in which they were originally found.

All of these pollen-tube genes were obtained in the progeny of irradiated plants. Most of them came from plants in which the parent pollen had been subjected to radium treatment. Their presence could be recognized by abnormalities in pollen-tube growth whenever the pollen of a carrier plant was tested, though proof of their egg-transmissibility required tests of their offspring. The five genes selected were chosen because of their variety as pollen-tube abnormalities and because of the distinctive character represented by each type of abnormality (BUCHHOLZ and BLAKESLEE 1932a) and the consequent ease and certainty in their diagnosis.

Genes affecting pollen-tube growth do not necessarily come from the treatment of pollen. The gene *s-1* was not derived from pollen treatment, but from the treatment of an ovary 36 hours after pollination (12–14 hours after fertilization). The change represented by this gene was therefore induced in the zygote, probably before the fertilized egg had completed its first mitosis. We have also observed pollen-tube mutations in the progeny of treated pollen tubes (BUCHHOLZ and BLAKESLEE 1930), in the progeny of seeds treated with X-rays, and in plants resulting from the treatment of flowers after fertilization. It may be recalled that tricarpel (*tc*) came from the progeny of a haploid plant, so that the origin of this gene was not in any way connected with radiation (BUCHHOLZ and BLAKESLEE 1927).

Our purpose in the rather intensive study of these pollen-tube genes was to establish their mode of inheritance and to discover their location on particular chromosomes. It is likely that genes of this kind may have an important role as a type of lethal in plants and their better appreciation may serve to throw much light on the nature of certain kinds of sterility and, more particularly, on the role of this mechanism of gametophytic selection in the evolution of plants.

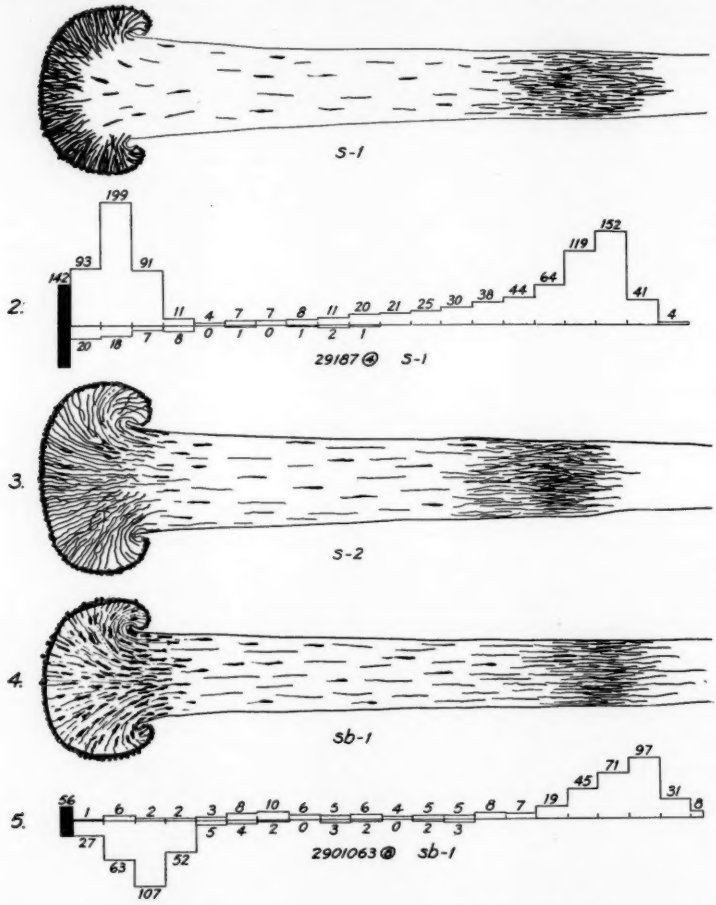
DESCRIPTION OF THE POLLEN-TUBE TYPES

The gene *s-1* (short pollen tube) was the first which we used extensively in crosses in an effort to discover linkages. In a carrier plant it is a recessive so far as the effect on the sporophyte is concerned. All carriers are fully as vigorous as non-carriers, and the pollen of a plant which carries this gene is perfectly normal in appearance as it is shed from an anther. There is no abnormality visible in meiosis, where the usual 12 bivalent chromosomes may be observed in diakinesis. It is only when the pollen is used in the pollination of a pistil that the pollen-tube peculiarity becomes recognizable. Six or more hours after pollination one finds the stigma penetrated for a short distance by a large number of very short pollen tubes, which usually range between $\frac{1}{2}$ and 2 mm in length. These remain short and do not function in fertilization, while the pollen tubes carrying the normal allelomorph of this gene have grown out long and continue growing through the style at the same rate as the pollen tubes of any normal 2n plant. Frequently the cell wall of the short tube is abnormally thick and transparent, the tubes are relatively broad, and usually do not burst. If the tests are conducted for 10–18 hours at 18–21°C the tips of the long normal pollen tubes are still to be found within the style, and if the pollen tubes in test slides are counted, a close approximation to a 1:1 ratio is found for these two kinds of gametophytes, long and short tubes. Figure 1 represents diagrammatically the appearance of a stigma with the short pollen tubes, and figure 2 shows a diagram of the pollen-tube distribution, which may be described as type V distribution (BUCHHOLZ and BLAKESLEE 1932a).

This gene will also manifest itself if the pollen taken from a *Datura stramonium* carrier is grown in the style of another species. An account of the behavior of pollen tubes in reciprocal pollinations between all possible pairs of ten species of Datura has been given elsewhere (BUCHHOLZ, WILLIAMS and BLAKESLEE 1935). The gene *s-1* may be recognized on the stigma and style of any species of Datura in which the pollen tubes of *D. stramonium* show a reasonable amount of growth. Even when the longer pollen tubes all burst, as is true in many of the interspecific pollinations such as *D. innoxia* pollinated with *D. stramonium*, the short pollen tubes with thick transparent cell walls and relatively broad diameter may usually be identified without difficulty.

Furthermore, we have pollinated *D. stramonium* plants carrying *s-1* with the pollen of *Datura quercifolia*, *D. ferox*, and *D. discolor*, and reared the hybrids. In half of these F_1 hybrids, one could easily recognize the presence of this *s-1* gene when tests of the pollen tubes of the hybrids were made, whether the tubes were grown in their own pistils or in the pistils of either parent. Even though a large proportion of the pollen of these

crosses consists of shriveled pollen grains, the good pollen which is present segregates for *s-1* pollen tubes and other longer tubes. This gene, there-



FIGURES 1–5.—Diagrams showing pollen tubes in styles with stigmas at left. Also graphs of distribution of pollen-tube ends. Figure 1—Diagram of pollen tubes of carrier of gene *s-1;* Figure 2—graph of distribution of ends of pollen tubes of above; Figure 3—diagram of pollen tubes of carrier of *s-2;* Figure 4—diagram of pollen tubes of carrier of *sb-1;* Figure 5—graph of distribution of pollen tubes of above.

fore, stubbornly retains its identity and its outstanding characteristics in a variety of interspecific hybrid combinations, as well as in crosses between genetic strains within the *stramonium* species.

The record of its egg transmission during six generations is as follows: 358 non-carriers and 336 carriers among the 2n offspring of 2n carrier parents; 115 non-carriers and 119 carriers among the 2n offspring of 2n+1 carrier parents; 30 non-carriers and 45 carriers among the 2n+1 offspring of carriers. The ratios obtained were all disomic since the locus of the *s-1* gene was not borne by the extra chromosomes in any of the 2n+1 parents tested. These records include both selfs and female backcrosses and give a total of 503 non-carrier and 500 carrier offspring, which is the 1:1 ratio expected on the assumption that the gene does not adversely affect viability. A male backcross of a carrier to a normal female produced 47 offspring, none of which were carriers. This confirms the conclusion reached from the behavior of the carrier pollen tubes that the gene cannot be transmitted through the pollen. Because of this non-transmissibility through the male, selfs and female backcrosses give similar ratios.

Another gene very similar and almost as characteristic as the one described above is *s-2*, which also gives a type V pollen-tube distribution. It was obtained from a plant in the immediate progeny of pollen which had been treated with radium. The short pollen tubes of *s-2* are somewhat longer than those of *s-1;* they are more slender back of the end and somewhat irregularly club shaped, occasionally somewhat irregular or beaded near the end, as shown in figure 3. They do not have the conspicuous thick wall described for *s-1*. They are likely to burst after longer periods, but very few will burst within about 12 hours; not all of them will burst after prolonged periods of testing. We have found no external structural differences between the carrier plants and the non-carrier sibs. So far as its effects upon the external appearance of the plant is concerned, therefore, this gene acts like a recessive. It has been noted in many plantings, however, that the non-carriers tend to come into flower a few days earlier than the carriers, so that the effect of this gene on a sporophyte carrier may be physiological. When considered in this light, therefore, it may be looked upon as slightly dominant, since this physiological effect may be more or less detectable in a heterozygote.

The gene *s-2* may be present in a plant without giving the short pollen tubes in every test. Early flowers of such plants, when their pollen was tested, have been found to show half of the pollen ungerminated (type VII distribution); then a week later the same plants were found to give a high proportion of germinated pollen with the characteristic short tubes. However, the short tubes or ungerminated pollen never give normal growth or long pollen tubes under any conditions of testing. We must conclude that *s-2* is a gene which has more than one method of expression and test slides must be examined with care (and the tests giving type VII distribution repeated) lest a carrier be incorrectly diagnosed. The 50 per

cent ungerminated pollen (type VII) is not to be considered a positive test for this gene. Causes other than genes may at times be responsible for poor pollen germination. If *s-2* is present in a plant, the pollen of another flower collected at a later time will usually give a positive diagnosis, unless the carriers suffer from drought or some other adverse environmental condition.

The gene *s-2* has also been recognized in the pollen of hybrids between *D. stramonium* and *D. ferox*, *D. quercifolia*, and *D. discolor*. If the tests are made at times when the pollen of a carrier gives satisfactory tests on styles of *D. stramonium*, the gene may be recognized when this pollen is grown in the pistils of other species.

The segregation records of *s-2* through egg transmission seem to vary. The first segregating planting from a self of the original parent gave 15 non-carriers and 17 *s-2* carriers. In the progeny of these carriers there were plantings in which the ratio was 62 non-carriers: 36 *s-2* carriers, and 32 non-carriers: 13 *s-2* carriers; the massed data (from 22 plantings with records on more than 6 plants up to 1935) gave 334 non-carriers: 228 *s-2* carriers among 2n plants. It is not unusual to have recessives run low in ratios due to their deleterious effect on viability and this would seem to be the case in the present instance. However, the ratios may have been affected by the method of sampling. We have found that the earlier flowering plants were more often non-carriers and those flowering a little later had higher proportions of carriers. It is possible that the unequal ratios of the above plantings may have been due in part to plantings made from incomplete seed germinations or to incomplete records of plantings where only a portion of the family was tested. In our latest stock planting (3401556), backcrossed 3 generations and 5 generations removed from the origininal plant, we had only 15 plants and 9 of them were *s-2* carriers, so that so far as this small number may indicate, there seems to be no shortage of *s-2* carriers. However, in all *s-2* plantings of 1935 combined, in which only a few plants in each planting were tested, the ratios recorded were 62 non-carriers: 41 carriers. Plantings with less than 6 plants determined were not included.

Another gene which we have recognized with ease in carrier plants is *sb-1*, which gives a type of pollen-tube distribution called VIa (BUCHHOLZ and BLAKESLEE 1932a) shown in figure 5. This gene gives short pollen tubes which burst within the region from 2–8 mm from the top of the stigma. The bursting is somewhat characteristic. The ends of the burst pollen tubes appear ragged or frayed, as if to suggest that the protoplasm is thin and flows up and down with ease along the surrounding elongated cells of the conducting tissue. Thus the burst ends have slivers or barbs extending forward and backward as shown in figure 4. The *sb-1* pollen tubes are al-

most never swollen or club-shaped, nor do they have a thick transparent wall as described for *s-1*.

We have attempted to recognize this gene when the pollen of a carrier is tested on the stigmas of other species. Grown in the styles of species which do not give bursting of the *D. stramonium* pollen tubes, it may be recognized, but on the pistils of *D. innoxia* and *D. meteloides*, where nearly all *D. stramonium* pollen tubes burst near the stigma, one could not be certain of recognizing this gene. Likewise, in making tests for carriers among interspecific hybrids it would be very difficult to recognize the gene *sb-1*, since there are usually many burst pollen tubes of all kinds from the pollen of such hybrids.

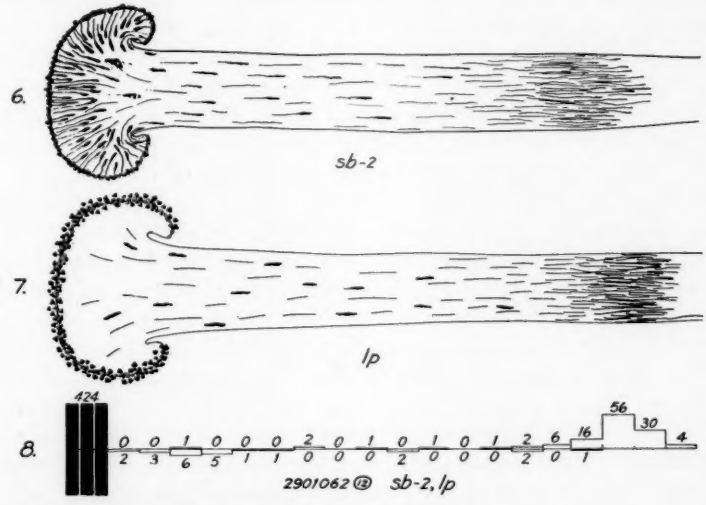


FIGURE 6.—Diagram of pollen tubes of carrier of gene *sb-2*.
FIGURE 7.—Diagram of pollen tubes of carrier of gene *lp*.
FIGURE 8.—Graph of distribution of ends of pollen tubes of a carrier of both genes *sb-2* and *lp*.

Our segregation records of *sb-1* show a ratio of 1 non-carrier to 1 carrier in female backcrosses. For the 2n carriers pollinated with pollen of various genes and with the pollen of 2n+1 plants, the massed records of 7 progeny plantings have given 236 non-carriers: 234 carriers. The offspring of 2n+1 carriers which had been pollinated with pollen containing various genes or "prime types" have shown 115 non-carriers: 105 carriers in 11 plantings. The locus of the gene was not borne by the extra chromosome in any of these 2n+1 parents. The totals for these female backcrosses are 351 non-carriers: 339 carriers. The records do not include many small plantings, in which only a few plants were tested.

One of the more difficult to recognize, yet distinctive pollen-tube genes is *sb-2* (fig. 6). The pollen of carriers gives either a type VII or a type VIa distribution (BUCHHOLZ and BLAKESLEE 1932a). The bursting of pollen tubes carrying this gene is preceded or accompanied by a swollen or relatively large club-shaped end, and occurs in the region of the stigma. In fact, not all pollen tubes which carry the gene will burst; they may only stop growing and may remain abnormally swollen. The appearance of *sb-2* is very similar to the abnormal tubes obtained when the largest chromosome 1·2 is present as an extra (BUCHHOLZ and BLAKESLEE 1932). The difficulty offered by this gene comes from the fact that very often the pollen grains which carry *sb-2* fail to germinate, so that we may have the 50 per cent ungerminated pollen (type VII) as the characteristic of this gene for many weeks during the early part of the reproductive cycle of a plant. Plants beginning to flower in July usually do not show the swollen or burst pollen tubes for several weeks, giving type VII distributions instead of VIa, and in dry years it is not positively identifiable until past the middle of August. Very late in the season (September) the pollen of *sb-2* carriers may give the 50 per cent ungerminated condition again.

While we have had interspecific hybrids which were doubtless carriers for *sb-2* it would not be an easy task to identify this gene with certainty. As stated before, both ungerminated pollen grains and burst pollen tubes are to be expected normally in the pollen produced by a hybrid, and this gene could only affect the quantitative relations of these abnormal classes of gametophytes. Of course, a gene whose diagnosis is not easy within the species would be expected to offer difficulties in the hybrids with other species.

We should state that *sb-2* was first recorded as a gene giving 50 per cent ungerminated pollen. We happened to make a routine cross of an *sb-2* carrier plant with a white flowered type, planted the F_1's and selfed and backcrossed them to white. When seeds from some of these were planted, the linkage with *p* was apparent in selfs and male backcrosses. When pollen-tube growth in the carrier plants was studied late in August, the characteristic which would most certainly identify this gene was noted as a type VIa, or short burst pollen tubes, rather than the 50 per cent ungerminated pollen of type VII. The identity of this abnormality as supplying the pollen tube disturbances which give aberrant ratios in the segregation of *p* could be established beyond question.

The origin of *sb-2* was from the treatment of pollen with radium. The first of the carrier plants, plant number 2901062(12) was one in the immediate progeny of the treated pollen. The distribution of the pollen tubes of this plant is shown in figure 8, a type VII distribution, with an excess of ungerminated pollen. This was also a carrier for *lp*, a gene to be described

below, and the fact that about three-fourths or more of the pollen was ungerminated suggested at once that this plant was heterozygous for two or more genes.

The segregation records in 12 plantings of selfs or female backcrosses in which six or more plants were tested have shown 100 non-carriers and 87 carriers.

Lobed pollen (*lp*) is a gene which occurred in plant number 2901062(12) that is, it was found in the same original plant which carried *sb-2*. Its characteristic feature is that the half of the pollen which remains ungerminated becomes swollen at the three germ pores of each pollen grain so that the ungerminated half of the pollen may be described as "lobed pollen" (fig. 7). We have called this a type VIIb distribution. This gene type was isolated for study because of the very unusual condition by which it may be recognized, and as one which gives a positive diagnosis for type VII which is characterized by 50 per cent ungerminated pollen grains. Sixteen plantings from carrier parents have given offspring with 169 non-carriers and 168 carriers.

This gene may be recognized in the pollen of hybrids made by pollinating a *D. stramonium* carrier with the pollen of another species. Approximately half of the hybrids will be carriers. The lobing is not always as pronounced, but it is an unmistakable condition, so that the *lp* type appears to have the same general stability as some of the other easily recognized gene types, *s-1* and *s-2*.

While *sb-2* and *lp* came originally from the same parent plant, these genes appear to be on different chromosomes. More recently we have obtained from the progeny of the same parent, a third unnamed gene, characterized by pollen tubes of the type VI which bursts within the upper half of the style. The new gene appears to have been carried along with, and partially masked by *lp* in many of our plantings.

METHODS USED IN LOCATING POLLEN-TUBE GENES IN PARTICULAR CHROMOSOMES

In attempting to discover which of the twelve pairs of chromosomes carry the different genes affecting pollen-tube growth, we found that we were more limited in our methods than with ordinary genes which are pollen-transmitted. These genes are egg-transmitted to half of the progeny only, so that tests to identify plants which carry the particular pollen-tube abnormality are necessary in each succeeding generation. A number of special methods for locating genes in particular chromosomes have been developed in connection with the Datura investigations (BLAKESLEE 1931). For the present problem four methods seemed available, although some of them could be used only in part.

Three of these methods are independent of a known gene in the chromosome tested and the other method depends upon the previous location of a gene in the chromosome. These methods are: (1) linkage with located genes; (2) linkage with such "prime types" as can be identified in the heterozygous condition by definite proportions of aborted pollen; (3) trisomic ratios, a method which is limited to the chromosomes which may be transmitted through the pollen as extras (11·12, 13·14, 15·16, 21·22, and 1·2 by way of 2·2); (4) hereditary behavior in transmission from a secondary trisomic to its primary. While we have made some use of all four of these methods, this last method was limited to the 1·2 chromosome.

Linkage with located genes

The method of linkage with known marker genes will be described first; and *sb-2* will furnish an illustration of how such linkages may be recognized.

TABLE 1

Offspring of parents heterozygous for pollen-tube gene (sb-2) and gene for white flowers (p).

♀ PARENTS	♀ BACKCROSSES TO WHITE (p)		♂ BACKCROSSES TO WHITE (p)		SELFS	
	PURPLE	WHITE	PURPLE	WHITE	PURPLE	WHITE
Non-carriers						
$(Sb\text{-}2)_2$ in pedigree 31091	36	43	92	90	240	87
Calculated	*39·5*	*39·5*	*91*	*91*	*245*	*82*
Carriers						
(*Sb-2 sb-2*) in pedigree 31091	239	215	185	700	287	242
Carriers in pedigree 3201679			188	642		
			373	**1342**		
Calculated for no linkage	*227*	*227*	*857·5*	*857·5*	*397*	*132*

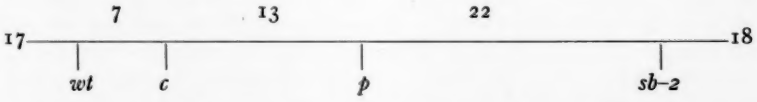
An *sb-2* carrier plant was pollinated with white (*p*). In the F_1 plants following this cross the *sb-2* carrier plants were identified. These were all heterozygous for white and were used in making male backcrosses to the recessive white. If the locus for this gene proved to be in the same chromosome as that for white (*p*) it would be in the chromosome carrying the dominant *P* and not in the chromosome carrying the allelomorph *p*. In male backcrosses, consequently, the *P* gametes, which also carry *sb-2*, would be eliminated during pollen-tube growth. The progeny would be all white except for the crossovers, which are purple. Table 1 shows the results for both male and female backcrosses and selfs.

There are actually a considerable number of purple plants or crossovers. Since only two classes appear, non-crossover whites and crossover purples, the percentage of crossing over is read directly. For *sb-2* and *p* it is close to

22 per cent. In practice we use the *sb-2* carriers in male backcrosses, which give the ratio of 373 purples to 1342 whites. If the non-carriers of *sb-2* are used in similar male backcrosses the ratio of purple to white is very nearly 1:1, our records for sib non-carriers being 92*P*:90*p*.

Linkage is seen also in the results from selfing which give 287 purples to 242 whites when 397 purples to 132 whites are the calculated values for a 3:1 ratio. The ratios from selfing indicate a lower crossing over value than the more accurate index from the male backcrosses. The non-carrier sibs when selfed have given 240 *P*:87 *p*, which is a proportion well within the probable error limits for a 3:1 ratio. When carriers were female backcrossed, the offspring showed a 1:1 ratio for purple and white without apparent effect of linkage. If the plants had been tested for pollen-tube growth, however, a higher proportion of purples than of whites would have been found to be carriers.

The *p* gene is in the 17·18 chromosome, as also the genes curled (*c*) and wilt (*wl*). It is also known that *c* and *wl* are in the ·17 half of the chromosome while *p* is in the ·18 half (BLAKESLEE 1929, 1930). The relative positions of these three genes on the 17·18 chromosome have been determined by their linkage relations (unpublished data, BLAKESLEE and AVERY). The gene *wl* in the ·17 half is about 7 units from *c*, and *p* in the ·18 half is about 13 units from *c*. To this linkage group we may now add the gene *sb-2* toward the end of the ·18 half. The data in table 1 indicate that *sb-2* is 22 units from *p*. Male backcrosses of *sb-2* carriers heterozygous for *c* gave 111 *C* to 205 *c* plants in the offspring, a proportion which indicates about 35 per cent crossing over between these two genes. A test with *wl* and *sb-2* showed only 33 per cent crossing over when somewhat more would have been expected, although there was a total of only 109 offspring in the backcross. The data as they stand are in general agreement with the arrangement of these four genes shown in the provisional map of the 17·18 chromosome given below.



The above method of linkages with various marker genes of known location was also used with the other pollen-tube genes. In many cases, carriers were pollinated with the pollen of plants homozygous for several marker genes in order to obtain F_1's heterozygous for both the pollen-tube gene and the markers. Male backcrosses were then made to the individual markers. Since no linkages were found for the other pollen-tube genes with the exception of the gene *s-2*, only this latter need be considered. Bronze (*Bz*),

a dominant character, and ferox white (*fw*) are markers in the 3·4 chromosome. Male backcrosses of carriers heterozygous for these genes gave the following ratios: 30 *Bz*:35*bz* and 61 *Fw*:82*fw*. The gene for inermis (*a*) is in the 11·12 chromosome and the male backcrosses involving this gene gave the ratio 33 *A*:35 *a*. Male backcrosses involving curled (*c*), already discussed as in the 17·18 chromosome, gave the ratio 88 *C*:92 *c*. Male backcrosses involving pale-1 in the 21·22 chromosome gave the ratio 65 *Pl*:72 *pl*. Since the gene for tricarpel (*tc*), which is in the 15·16 chromosome, is transmitted poorly through the pollen, female backcrosses were used when this marker was involved and the *s-2* plants identified in the offspring by tests of pollen-tube growth. Among the *Tc* offspring the ratio was 31 *S-2*:21 *s-2* and among the *tc* offspring the ratio was 30 *S-2*:16 *s-2*. It will be remembered that the gene *s-2* runs low in female backcrosses. None of the above tests with gene markers showed any linkage. By this time, it had been determined by another method that the gene *s-2* is located in the 13·14 chromosome. Linkage tests were therefore made with albino-2 (*al-2*), which is a marker for this chromosome. For albinos the male backcrosses to the recessive are not practical without a supply of flowering albino branches grafted on normal stocks. In consequence, the linkage was calculated from segregation from self pollinations.

The pollen of a plant heterozygous for *al-2* was used on a heterozygous *s-2* female and in the progeny only the selfed seeds of *s-2* plants were saved and planted. The progenies of four of these *s-2* plants did not segregate for albino and were discarded, those of three of these gave albino segregation in the combined proportion of 260 normal: 134 albino, or 34 per cent albino. This ratio shows an excess of albinos. The calculated values for a 3:1 ratio are 295.5 *Al*:98.5 *al*, so that the excess of 9 albino plants per 100 is attributable to this linkage. The linkage value may be computed from the selfed progeny in this case with a fair degree of accuracy since there are only two classes of phenotypes, resulting from the two classes of gametes carried by the functional pollen tubes. The male non-crossover gametes all carry albino and only the crossover gametes carry the normal allelomorph of albino. If there were no linkage, the expected proportion from selfing would be 25 per cent albinos and if the linkage were close, let us say with less than one per cent crossing over, the expected proportion would be close to 50 per cent albinos. Thus the linkage scale (from 1–50 per cent crossovers) is read as the difference obtained between the limits of 50 per cent albino seedlings and 25 per cent albino seedlings. Since the increases in linkage values decrease the observed proportion of albino seedlings, the linkage scale reads in reverse order from the seedling scale.

Our observed albinos form 34 per cent, or 9 per cent in excess of the 25 per cent expected from selfing when linkage does not occur. This 9 per

cent is nearer to the 25 per cent limit than to the 50 per cent limit on the scale and must be multiplied by 2 and subtracted from 50 in order to give the linkage value which would be expected in a male backcross. Our observed results, therefore, indicate a crossover value of 32 per cent between *s-2* and *al-2*, assuming that there are no other conditions present which would modify this ratio. The situation may perhaps be made clearer by giving the ratio of *Al* and *al* for the male and female gametes.

Female gametes—1 *Al*+1 *al*

Male gametes—32 *Al*+68 *al*

F_2 zygotes—32 $(Al)_2$+100 *Al al*+68 $(al)_2$ or 132 *Al*:68 *al*.

The ratio 132 *Al*:68 *al* equals 260 *Al*:135 *al*, which, except for a discrepancy of 1 due to disregarding decimals, are the numbers actually found in the backcross.

Linkage with prime types

The method of linkage with appropriate prime types has been used successfully for the location of the gene *s-2*, as also for the location of other genes in Datura (BLAKESLEE 1931). Prime types (BERGNER, SATINA and BLAKESLEE 1933), it will be remembered, are races in which certain chromosomes have been modified in terms of those of our standard Line 1. They include chromosomal changes brought about by segmental interchange and simple translocation, which give circles, chains and "neckties" of four or more attached chromosomes in the heterozygous condition. Most of the "neckties" are associated with 50 per cent aborted pollen grains some of the chains with 50 or 25 per cent aborted grains and a few of the circles with 25 per cent pollen abortion. The cause of the pollen abortion in some of these cases has been discussed (BERGNER, SATINA and BLAKESLEE 1933) but need not concern us here. The important fact for our present consideration is that plants heterozygous for such prime types can be identified by inspection of the pollen without resorting to cytological technique. Linkage of a gene with the modified chromosomes of such prime types can be used therefore to show that the locus of the gene in question is in one of its two or three modified chromosomes.

Our *s-2* carriers, which are in the standard Line 1, were used as females in crosses with the various prime type testers which show definite proportions of shriveled pollen grains in the heterozygous condition. Plants in the F_1 generation from this cross show the percentage of aborted pollen grains characteristic for the heterozygous prime type involved in the cross. An F_1 carrier was then identified and its pollen used in a male backcross to Line 1. Half of the male gametes will carry the Prime type chromosomes and half the unmodified Line 1 chromosomes. If no linkage is involved with *s-2* the Line 1 male gametes will unite with the Line 1 female gametes

to produce plants homozygous for Line 1 and characterized by good pollen. Prime type male gametes, on the other hand, will unite with the Line 1 female gametes to produce plants heterozygous for the prime type and characterized by a definite proportion of aborted pollen. The results of the tests are shown in table 2, where linkages with the chromosomes involved in the several prime types are shown by + signs and lack of linkage is shown by − signs.

TABLE 2

Male backcrosses showing segregation of male gametes in s-2 carriers heterozygous for prime types.
A + sign indicates linkage with prime type tester, a − sign indicates no linkage.

PRIME TYPE USED	TYPE OF ♂ GAMETES L 1	PT	CHROMOSOMES TESTED BY BACKCROSSES 1·2	3·4	5·6	7·8	9·10	11·12	13·14	15·16	17·18	19·20	21·22	23·24
3	37	40						−					−	
7	40	31					−					−		
11	4	79						+	+		+			
15	24	24		−						−				
16	42	44				−							−	
17	3	63					+		+					+
19	8	74		+					+					
30	3	106							+					+
34	74	75						−						−
36	25	73			+				+	+				

Prime type 3 has the chromosomes 11·12 and 21·22 modified. Prime type 7 has the chromosomes 9·10 and 19·20 modified. Backcrosses involving these two prime types give a 1:1 ratio of plants with good and with a characteristic percentage of aborted pollen and hence show that *s-2* is not linked with the modified chromosomes involved in these two prime types. The case is quite otherwise with prime type 11 which contains chromosomes modified from the 11·12, 13·14, and the 17·18 chromosomes. Out of the 83 plants from the backcross, 79 had 25 per cent aborted pollen and thus were shown to have come from prime type 11 male gametes while only 4 had good pollen and were thus known to have come from Line 1 male gametes. The gene *s-2* was originally in Line 1 and hence linked to Line 1 chromosomes. Unless the linkage is broken by crossing over, the Line 1 pollen of the F_1 plants will contain the chromosome bearing the *s-2* gene, so that their pollen tubes are kept back or sifted out when this pollen is used in a backcross. It is only the prime type 11 half of the pollen which remains functional and reaches the ovary. The progeny from this male backcross to Line 1, therefore, will be the result of the cross Line 1 by prime type 11 and all the plants will be recognizable as

heterozygotes for prime type 11 by 25 per cent aborted pollen grains except for a few crossover plants which will have good pollen. As seen by the table, there were 4 or 4.8 per cent of such crossovers between *s-2* and prime type 11. It is thus shown that *s-2* is linked with prime type 11 and hence its locus is in one of the three chromosomes which have been modified in this prime type. In other words, its locus is in the 11·12, the 13·14, or the 17·18 chromosome. Linkage is shown again in tests with prime type 17 which enables us to say that *s-2* is located in the 9·10, the 13·14, or the 23·24 chromosome. It must therefore be in the 13·14 chromosome, which is the only one involved in both prime type 11 and prime type 17. As will be seen from the table, this conclusion is confirmed by tests involving four other prime types which contain modified 13·14 chromosomes. Prime types not involving the 13·14 chromosome do not show linkage with *s-2*. Negative evidence is not conclusive since the distance of the gene may be so far removed from the point which limits the linkage that 50 per cent crossing over may occur; and this cannot be distinguished from random assortment of unrelated chromosomes. All the chromosomes have been tested by the prime type method except the 1·2 chromosome and this chromosome has been tested by the trisomic method to be discussed later. In using the prime type method it is immaterial whether the F_1 is male backcrossed to Line 1, which was the usual procedure, or to the prime type. Either female parent would serve to identify the male gametes. In the first case the plants with good pollen would have come from Line 1 male gametes and those with definite proportions of aborted pollen from the prime type male gametes, while in the second case it would be the plants with good pollen that came from the prime type male gametes. In a few cases, such as prime type 34, the homozygous prime type is recognized by morphological peculiarities, a fact which enables one to record by inspection of the plants rather than by examination of the pollen when the prime type is used in backcrosses.

Tests by the prime type method are being made of the other pollen-tube genes that we have discussed in this paper. The results so far obtained appear to indicate that *s-1* is in the 9·10 or the 19·20 chromosome.

Method of trisomic ratios

From the beginning of our studies of the pollen-tube genes we have attempted to use trisomic ratios as a means of locating these genes. It may be recalled that for the gene tricarpel (*tc*) the trisomic method was used successfully (BUCHHOLZ and BLAKESLEE 1927), but in that case the 2n+1 plants could be rendered heterozygous for *tc* by direct transmission of this gene through the pollen of the homozygotes. Although *tc* suffers a high elimination in pollen transmission when it is obtained from a hetero-

zygous plant and some even when it is homozygous, the F_1's may be obtained without great difficulty and the trisomic segregation may be observed in female backcrosses without the complications of the disturbance due to differential elimination of *tc* pollen tubes in male backcrosses.

However, the pollen-tube genes described in this paper cannot be transmitted through the pollen and their homozygotes are unknown. Our only practical method of combining 2n+1 plants with the pollen-tube genes is to obtain carriers in crosses resulting from the successful pollen transmission of the extra chromosome.

We have indicated the possibilities of pollen transmission of extra chromosomes in previous papers (BUCHHOLZ and BLAKESLEE 1932, BLAKESLEE 1934). Special methods must be used, such as restricting the pollen and selecting the seeds from the lower half of the seed capsule. Where restricted pollinations were made, usually the lower half contained the largest proportion of trisomics. In some instances we mixed the pollen of three or four trisomic plants and used this pollen mixture sparingly in pollinations with the expectation that more than one of the 2n+1 types used for the pollen would appear in the offspring. We have also transmitted extra chromosomes by a method of splicing styles (BUCHHOLZ, DOAK and BLAKESLEE 1932), but this method had not been perfected when we began the work reported in the present paper. From our efforts at pollen transmission with restricted pollinations we have been able to obtain 2n+1 plants for the primary chromosomes 11·12, 13·14, 15·16, 21·22, and for the secondary chromosome 2·2. The latter may be used in obtaining its primary 2n+1·2 as a carrier. Thus we should be able to test for trisomic segregation from five of the twelve chromosomes.

The segregation for *s-2* from three of the trisomics is shown in table 3. The numbers tested from the 2n+15·16 were too small to be included and the 1·2 chromosome was handled by another method to be discussed shortly.

Had we relied on the trisomic methods alone, we would have failed to locate *sb-2* since this gene is on the 17·18 chromosome which is not pollen transmissible as an extra. Although we stated (BUCHHOLZ and BLAKESLEE 1932) that this extra chromosome gives indications of being pollen transmitted at times to a very limited extent, we have been unsuccessful thus far in several attempts to obtain 2n+17·18 carriers of *s-1*, *s-2* and *sb-1*.

As our table 3 indicates, the segregation obtained from 2n+1 carriers was 1:1 and did not differ essentially from the segregation of ordinary 2n plants except in the case of the 2n+13·14 carrier. Here the expectation of a trisomic ratio of 2:1 among the 2n offspring and 1:2 among the 2n +1 offspring is closely approximated. The evidence from trisomic ratios,

therefore, confirms the evidence from linkage with prime types and from linkage with the visible character *al-2*. There seems little doubt that the gene *s-2* is in the 13·14 chromosome.

TABLE 3

Segregation from 2n+1 types heterozygous for s-2.

♀ CARRIERS USED IN CROSSES	OFFSPRING				NO. PLANTS NOT TESTED		NO. PLANTS GROWN
	2n		2n+1				
	NON-CARRIERS	CARRIERS	NON-CARRIERS	CARRIERS	2n	2n+1	
2n+21·22	36	29	3	1	8	7	85
2n+13·14	45	24	3	6	7	4	89
2n+11·12	26	21	12	6	1	1	67

We have found that the trisomic method is not a very practical one for the initial location of one of these pollen-tube genes. This method depends upon the distinction between a 2:1 and a 1:1 ratio and thus requires large plantings. Every plant moreover should be tested for abnormalities of pollen-tube growth, and this procedure entails a relatively large amount of labor in comparison with the other methods available.

Segregation from secondaries

A fourth method which was used is that of testing the inheritance of the pollen-tube gene from a secondary to its primary. The primary 1·2 chromosome is not pollen-transmissible, but the secondary 2·2 may be easily obtained by pollen transmission from restricted pollinations. An *s-2* carrier plant combined with a 2n+2·2 plant may be expected to give among the primaries which it throws some *s-2* 2n+1·2 plants. If all 2n +1·2 plants so obtained are *s-2* carriers without exception, it might indicate that the gene is on the 1·2 chromosome. Our tests by this method were all negative; there were both carriers and non-carriers of *s-2* among our 2n+1·2 plants coming from 2n+2·2 carriers. We could therefore conclude that, barring the possibility of crossing over on the ·2 half of this chromosome, the genes in question were not in 1·2. Since crossing over might occur between the extra 2·2 and a gene located on the ·2 half of the 1·2 chromosome, this method would be a certain test only for a gene located on the ·1 half of this chromosome, if the tests were repeated for at least ten to twelve different 2n+1·2 plants reared from 2n+2·2 carriers and these were found to be carriers without exception.

In the experiments which had for their purpose obtaining 2n+1 types heterozygous for the pollen-tube genes, some additional data on transmission of extra chromosomes through the pollen were secured. Thus,

when a normal plant was crossed with a 2n+2·2 male parent and a limited amount of pollen was used, a relatively high proportion of 2n+2·2 plants (18.7 per cent) were secured in the offspring. The high transmission of the 2·2 chromosome through the pollen is more striking when the progenies of seeds from the upper half of the capsule are considered separately. From the upper half of the capsule there was only one 2n+2·2 plant from 41 seedlings while from the lower half there were 16 (or 32 per cent) 2n+2·2 plants from 50 seedlings. The experience with this, as well as with the other 2n+1 types, the extra chromosome of which can be carried through the pollen, shows that the pollen-transmission of the extra chromosome can be depended upon to yield a sufficiently high proportion of 2n+1 plants to secure some trisomic carriers.

DISCUSSION

While these pollen-tube genes were all obtained in the progeny of irradiated plants, there is no reason to suppose that similar abnormalities might not arise spontaneously. If they should arise they would soon be lost in the gametophytic selection which purges the pollen in each generation. Pollen-tube genes would be transmitted through the eggs of a carrier to half of the progeny in each generation. In seven generations there would be less than 1 per cent carriers in the total progeny of a carrier plant. This fact, added to the probability that their spontaneous occurrence would probably be infrequent, would make the search for spontaneous mutations of this kind in inbred lines very discouraging from the start. We have not found genes of this kind in untreated materials, but we have not made a systematic search for them on a large scale. Our examination of hundreds of plants in various investigations has not led to the discovery of any of the pronounced and easily recognized genes of the category described here.

We have also examined the progeny of irradiated plants which, in spite of the treatment, gave normal pollen-tube growth, and we have found that such progenies had essentially normal pollen-tube growth in the subsequent generation. When pollen-tube genes of any type were first recognized in the progeny of treated plants they were usually found to segregate approximately in a 1:1 ratio through egg transmission, though the carrier plants were sometimes deficient. We have also observed one very pronounced pollen-tube abnormality which was not transmitted maternally.

As stated in our introduction, there is no connection between these pollen-tube abnormalities and the manner in which the irradiation was administered. The pollen-tube genes are not peculiar to pollen treatment. Abnormalities of various types have appeared in the progeny of irradiated seeds and of plants otherwise subjected to the action of radium and

X-rays. However, when treatment is applied to seeds and more mature plants in which there are already several cells in the meristematic stem tip, the various forks or branches of the plant which may develop subsequently often differ from each other. The flower bud examined cytologically is not necessarily the same as another one on the same plant used for a seed progeny. It is not certain that all parts of the same flower or seed capsule are genetically alike. It is only after one generation of seed progeny had been grown that the different meristematic growing points would be uniform so that one might advantageously begin in a series of analytical studies.

The peculiar advantage of pollen treatment as a source of these pollen-tube abnormalities, as well as other kinds of genes, lies in the fact that every branch of a plant in the immediate progeny, or all of its flowers, may be expected to be genetically similar. When pollen is treated, we may be sure that only a single treated cell, the sperm nucleus, contributes a haploid set of chromosomes and that there is a similar haploid set of untreated chromosomes contributed by the egg. Thus we not only save a generation in obtaining plants in which the cells are genetically uniform throughout, but we may observe the induced changes if any are present which show in pollen-tube growth, in the pollen of the immediate progeny of the cells treated.

When radium is used the treatment is very easily administered to the pollen, on account of the short distances of exposure to this agent; and with either the X-rays or radium the treatment may be given in a laboratory far removed from the plants which furnish or receive the pollen.

In spite of the very pronounced effect of the various genes described here on the pollen tubes which carry them they appear to have no effect on the segregation ratios of genes carried by other chromosomes. Of course, they operate to lessen the seed yield in the progeny, but this is partially compensated by the fact that pollinations are usually heavier than necessary to supply the pollen tubes needed in fertilization. It is only when they are found to be linked with genes of other types that they give rise to pronounced disturbances in genetic segregation ratios of the genes so linked. Since there are 12 pairs of chromosomes in Datura, the probability of linkage of any one of our pollen-tube genes with other genes would appear to be one in twelve. However, as is well known, linkage between genes with approximately 50 per cent crossing over would give the same results statistically as the independent assortment of non-linkage. The expectation of disturbances due to linkages of a random sample of other genes of unknown location with one of our pollen-tube genes would therefore be less than one in twelve. Our data on linkage studies have borne out this expectation.

EVIDENCE THAT POLLEN-TUBE ABNORMALITIES ARE DUE TO GENES

That the pollen-tube genes described in this paper are actually genes and not some other type of abnormality is indicated by several lines of evidence. (a) The pollen of these plants has no more shrivelled pollen grains than any other 2n plant. The pollen is normal in appearance as it is shed from the anthers. (b) The carrier plants have been examined by our group of cytological investigators (the first carriers as well as carriers four or more generations later) and have always been found to be normal at meiosis showing in diakinesis 12 bivalent chromosomes. (c) The genes follow a definite mode of inheritance, they are transmitted maternally only to half of the progeny. (d) They give segregation ratios which approximate the theoretical expectations which follow a gamete ratio of 1:1. The paternal segregation is recordable as the number of long normal and short abnormal pollen tubes, and the maternal segregation is recorded by the ratio of the non-carriers to the carriers, which is usually also a gametic 1:1 ratio. (e) They are like recognized genes for visible characters in that they may be located in definite chromosomes and show linkage phenomena with the accompanying crossing over. These pollen-tube genes, therefore, fulfill all of the usual tests for genes.

Some might suggest that these genes are deficiencies, deletions from chromosomes, or translocations. The fact that they are all recessives might also suggest deficiencies. If they are deficiencies the parts of the chromosome missing must be very small. Deficiencies involving only a half of a chromosome, or in some cases only the hump or satellite of a chromosome (BERGNER, SATINA and BLAKESLEE 1933) have been known to result in shrivelled pollen. However, the pollen grains which carry the pollen-tube genes appear to be perfectly developed. Furthermore, if the deficiency is large or if a translocation should be involved one might expect to observe this cytologically.

It should perhaps be mentioned that in certain cases in Datura (BLAKESLEE, AVERY and BERGNER 1935) excess chromosomal material has been found to behave in inheritance like a recessive and is distinguishable from a gene only by cytological examination. Recently, moreover, a pure-breeding type with extra chromosomal material has been synthesized (BLAKESLEE, BERGNER and AVERY 1936) which cannot be distinguished from the effects of a gene by the the usual cytological tests. We feel it desirable, therefore, to exercise a degree of caution in stating that we believe the pollen-tube abnormalities we have described are due to specific genes. The same caution, however, should be exercised regarding most other described genes.

SUMMARY

Five abnormalities in pollen-tube growth are described. Two (*s-1* and *s-2*) are characterized by very slow growth of tubes without bursting. Two (*sb-1* and *sb-2*) are characterized by slow growth with bursting. One (*lp*) has pollen grains which fail to germinate, but become lobed at the germ pores.

These abnormalities are not transmitted through the pollen but are transmitted through half the egg cells.

The pollen-tube type *sb-2* shows linkage with the genes *p*, *c*, and *wl* which have been previously shown to be in the 17·18 chromosome.

The type *s-2* has been located in the 13·14 chromosome (a) by linkage with certain prime types which show definite proportions of aborted pollen when heterozygous, (b) by trisomic ratios from 2n+13·14 plants which were heterozygous for *s-2*, and (c) by linkage with a gene (*al*) in the 13·14 chromosome.

The pollen-tube abnormalities are not associated with any recognizable chromosomal peculiarities, show linkage with known genes and are therefore believed to be due to genes.

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RECURRENT AUTO- AND EXOMUTATION OF PLASTIDS RESULTING IN TRICOLORED VARIEGATION OF HORDEUM VULGARE

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EXHAUSTIVE experiments conducted by Sô (1921) clarified the genetic nature of the white-striated 'Okina-Mugi', an agronomical variety of *Hordeum vulgare*, the results of which were referred to in one of my early publications (IMAI 1928). I have since verified his conclusions with hybridization experiments. In 1930, one of the variegated plants from a pure line showed a tricolored mosaic through additional coloration to yellow. Since the appearance of the tricolored stock I have extended my research in order to learn its nature. Last winter's cold severely affected and finally destroyed all the tricolored rosettes and some of their sister white-variegated ones, putting an end to the investigation to be here described.

The white-variegated barley behaves as a simple recessive, the variegation being linked very closely to the normal allele of contracted habit (Sô, OGURA and IMAI 1919). Although the gene itself is very constant, it stimulates the recurrence of plastid mutation from green to white, or in other words, exomutation of plastids occurs recurrently (IMAI 1936). The exomutation rate varies with environment (IMAI 1935). Since the white stripes or sectors are due to the propagation of mutant albinotic plastids, the white seeds give rise to albinos when they are germinated. In this plant the inheritance of plastids is maternal, the pollen tubes contributing no plastids to zygotes through fertilization.

In 1930, a white-variegated pedigree gave a seedling with green-variegated yellowish (tricolored) leaves along with 165 white-variegated (almost green at the seedling stage) sister seedlings. The mutant further grew to produce four variegated ears. The leaves of the adult plant are RIDGWAY'S Light Greenish Yellow, variegated with Spinach Green stripes as well as with white ones, presenting a tricolored foliage. The yellow color varies with the condition of the leaves, the young leaves resembling Cosse Green, which fades away to Martius Yellow with age. Because of the fact that the yellow parts become very greenish, although the white stripes are very evident, usually the difference in the color of the ears is not very marked.

From the 1931 sowing, the seeds germinated with results as shown in table 1, in which will be found the data for progenies of the mutant and its sister white-variegated plants.

In this and subsequent tables the almost green (pseudogreen) seedlings which develop into white-variegated are classified as white-variegated while the green-variegated yellowish (bicolored) seedlings which later develop white stripes are classified as tricolored.

TABLE 1

MOTHER PLANT	NUMBER OF PLANTS	WHITE-VARIEGATED	TRICOLORED	YELLOW	ALBINOTIC	MOSAIC	TOTAL
White-variegated	123	25407	—	—	811	31	26249
Tricolored	1	150	17	35	3	0	205

Except for the occurrence of a few albinotic and mosaic seedlings, the white-variegated sister plants bred true to type. The tricolored mutant gave 150 white-variegated, 17 tricolored, 35 yellow, and 3 albinotic seedlings, that is, 73.2 per cent reversional individuals. The yellow seedlings die about three weeks after germination. The tricolored seedlings, the extent of variegation of which varies in all gradations, survive and grow further, except however those having small green parts.

Seeds of 32 white-variegated and 6 tricolored plants derived from the mutant pedigree were sown in 1932. The data collected from the seedling beds are shown in table 2.

TABLE 2

MOTHER PLANT	NUMBER OF PLANTS	EARS	WHITE-VARIEGATED	TRI-COLORED	YELLOW	ALBI-NOTIC	MOSAIC	TOTAL
White-variegated	32	268	12444	—	—	508	8	12960
Tricolored	6	white-variegated. 30	1351	—	—	57	0	1408
		tricolored 10	360	40	36	4	1	441

Six tricolored plants, which were mosaic for green, yellow, and white, shot out 40 ears. Of these, 30 ears were white-variegated and produced 1351 white-variegated offspring, together with 57 albinotic seedlings, whereas 10 ears were tricolored and gave 360 white-variegated, 40 tricolored, 36 yellow, 4 albinotic, and 1 mosaic. The tricolored plants thus failed again to breed true to type, producing many reversional white-variegated in their offspring. The green stripes or areas that occurred in the tricolored sporophytes are due to somatic mutation, the occurrence of which was so frequent that all the yellow ears were variegated, the offspring containing many mutated white-variegated seedlings. Somatic mutation occurs at every stage of plant ontogeny, the earlier mutation resulting in heavily green-variegated plants that bear many green ears with white stripes, and later mutation in slightly variegated plants. Some yellow seedlings may therefore be variegated and others without any appar-

ent green stripes. Pure yellow seedlings, however, die before sending out second blades, without a chance to grow further by means of the mutated green tissues, which, if they had sufficiently developed at an early stage, would have made a full life cycle possible.

TABLE 3

	EARS	WHITE-VARIEGATED	TRICOLORED	YELLOW	ALBINOTIC	MOSAIC	TOTAL
Tricolored	4	171	38	37	0	0	246
Slightly tricolored	1	0	0	46	0	0	46

From white-variegated plants, 32 tests gave rise to 12444 white-variegated, 508 albinotic, and 8 mosaic seedlings. The recurrent mutation being of plastids, and not of genes, the reversional plants invariably bred true to white-variegated. The white-variegated shoots from tricolored plants behaved in the same way.

In 1933, the sowing was restricted to seeds collected from one tricolored plant, the others having been damaged by moths. The results obtained are shown in table 3.

The data confirm the mutable nature of the tricolor. The four ears that gave mixed progeny were variegated with green stripes, while the fifth

TABLE 4

YEAR	MOTHER PLANT	NUMBER OF PLANTS		EARS	WHITE-VARIE-GATED	TRICOL-ORED	YELLOW	ALBI-NOTIC	MOSAIC	TRICOL-ORED MOSAIC	YELLOW MOSAIC	TOTAL
	White-variegated											
1934		150		716	34249	—	—	1687	31	0	0	35967
	Tricolored	10	white-varieg.	8	423	—	—	14	0	0	0	437
			heavily tricol.	10	505	7	6	22	3	0	0	543
			tricolored	28	868	298	296	43	4	0	0	1509
			slightly tricol.	3	13	31	104	1	0	0	0	149
	White-variegated											
1935		30		162	3913	—	—	260	5	0	0	4178
	Tricolored	31	white-varieg.	84	2956	—	—	127	0	0	0	3083
			heavily tricol.	30	957	71	84	48	17	0	0	1177
			tricolored	74	1086	344	982	125	3	1	2	2543
			slightly tricol.	17	25	19	547	27	0	0	2	620

ear was very slightly variegated, giving however all yellow seedlings that died at the seedling stage.

The full data were obtained in 1934 and 1935 by successive pedigree cultures, as shown in table 4.

Tests made during these two years with offspring of 180 white-variegated derived from the tricolored mother plants, resulted in all producing the expected forms. The ears on 41 tricolored plants were classified according to the degree of variegation into 40 heavily tricolored, 102 tricolored, 20 slightly tricolored, beside 92 white-variegated. The last-named ears gave all pseudogreen (white-variegated) seedlings, except some albinos, while others segregated into yellow and its variegated forms.

Experiments and observations made during five years support the view of recurrent mutation of plastids as will be seen from the following results: First, crossing experiments conducted in 1935 revealed that tricolor variegation is transmitted only through the mother. The tricolored ears, when out-pollinated by self green, gave 32 white-variegated, 15 tricolored, 20 yellow, and 2 albinotic seedlings, while its reciprocal mating resulted in 82 normals. Except for the yellow and albinotic seedlings, they were trans-

TABLE 5

EARS	WHITE-VARIEGATED	TRICOLORED	YELLOW	TOTAL	ALBINOTIC
White-variegated	4730	—	—	4730	198
	100%	—	—	100%	—
Heavily tricolored	1465	95	90	1650	70
	88.8%	5.75%	5.45%	100%	—
Tricolored	2643	738	1388	4769	125
	55.4%	15.5%	29.1%	100%	—
Slightly tricolored	38	50	697	785	28
	4.8%	6.4%	88.8%	100%	—

planted to a field to facilitate further growth, but the severe winter killed these rosettes. Second, no ears were pure yellow, but variegated with green, indicating the recurrence of somatic mutation. If tricolor variegation was due to sorting out of mixed green and yellow plastids, pure yellow ears or tillers should have resulted, whereas scarcely any came under my observation, although a few leaves may have been yellow without green variegation. Third, too many tricolored seedlings were segregated to be regarded as the result of sorting out of mixed plastids. The green-and-white mosaic seedlings from white-variegated ears amounted to only 0.2 per cent, average (IMAI 1936), while the yellow-and-green (tricolored) seedlings were quite numerous (see tables).

The data on the offspring of the tricolored plants for five years have been summed up and shown in table 5. In the table, green-and-white mosaic, yellow-green-and-white mosaic, and yellow-and-white mosaic seedlings are included in white-variegated, tricolored, and yellow segregates, respectively. Somatic segregation is apparent from the data, while the extent

of variegation in the mother ears is strongly correlated with the proportion of the white-variegated (pseudogreen) and yellow offspring.

From the foregoing descriptions it is clear that tricolored barley is conditioned by the following gene and plastids: The gene, which is recessive to normal, stimulates the recurrence of plastid mutation from green to white and from yellow to white. The plastids are exomutable from green to white and from yellow to white, and automutable from yellow to green. The occurrence of the original tricolored plant is due to sporadic plastid mutation from green to yellow. The mutant yellow plastids, owing to their automutable nature to become green in sporophytic ontogeny, resulted in a bicolor. Further, through stimulation of the gene carried by the stock, recurrent exomutation of plastids added to it white variegation. The scheme presented in figure 1 indicates the action and reaction of the gene and plastids.

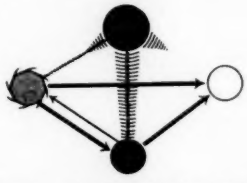

FIGURE 1.—The genetic mechanism of tricolored barley. The large circle is the nucleus and the small the plastids. The solid ripple-like lines indicate the action of the nuclear genotype in producing ample chlorophyll pigments in the plastids, while those extending only a short distance out from the centre of the nucleus represent the manner in which the plastid character manifests itself independent of the control of the normal nuclear genotype. The zigzag line joining nucleus and plastid indicates the effect of the former in changing the property of the latter. The solid plastid is green, the dotted yellow, and the blank white. The vanes of the plastid show automutability. The thick arrows indicate recurrent mutation and the thin, sporadic mutation.

Plastid exomutation from green to white is almost restricted to the post-embryonic stage (IMAI 1928, 1935), while conditions differ when automutation occurs from yellow to green. The occurrence of green-and-white mosaic seedlings is believed to have started from egg-cells containing mixed plastids, and not from early mutation. The segregates, namely, the yellow-and-green seedlings, however, are too numerous to be attributed to the same origin, so that the majority of them should be characterized by green variegation as the result of early somatic plastid mutation. On this basis, plastid automutation from yellow to green occurs at all stages of somatogenesis, including embryonic ontogeny.

SUMMARY

Since white-variegated barley is caused by a recessive gene that stimulates the recurrence of plastid mutation from colored to white, exomuta-

tion of plastids results in white variegation. Through sporadic plastid mutation, green changed to yellow. The yellow plastids being automutable, frequently change to green, so that green variegation occurs in the yellow foliage. Since both green and yellow plastids are controlled by the stimulating gene, they transform into white plastids. Therefore, white variegation occurs in green and yellow tissues. The inheritance of yellow and white plastids is non-Mendelian, being transmitted maternally.

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ON THE GENETICS OF THE SPOTTED PATTERN OF THE GUINEA PIG

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INTRODUCTION

COAT patterns in which color is restricted to spots on a white ground are extremely common in guinea pigs, and have naturally attracted the attention of geneticists from the first. That the genetic situation is somewhat complicated is indicated by the fact that there are at present at least three decidedly different interpretations.

Variations in this type of pattern, ranging from mere traces of white in a few locations (toes, nose, between ears) to black eyed white, through intermediate grades showing much diversity in localization and often extreme asymmetry, were described by CASTLE (1905), who, however, attempted no genetic interpretation. GOODALE and MORGAN (1913) published data from which they concluded that "the spotted coat is a very complex affair depending presumably on a number of factors." They suggested the hypothesis that there may be one recessive factor (ss) necessary for any spotting with extension due to other factors (s_1s_1, s_2s_2, s_3s_3 etc.). IBSEN (1916) provisionally treated spotting as due to a recessive factor s, but noted "evidence is accumulating which seems to indicate that the relationship is not as simple as has hitherto been supposed."

In a list of guinea pig color factors (WRIGHT 1916) the symbol Σw was used for the "assemblage of unanalyzed factors which determine white spotting." In a review in the next year, however, (WRIGHT 1917), a pair of alleles S, s was listed as differentiating at least certain inbred stocks. The extreme variability of the pattern in both amount and localization of white even after 16 generations of brother-sister mating was cited as evidence for an extraordinary amount of non-genetic variability and as the reason for difficulty in genetic analysis. An effect of sex on spotting was noted. Females of each of 23 inbred strains were found to have slightly more white on the average than males. An effect of s as a modifier of the character of the tortoiseshell pattern of black and yellow was noted.

Early crosses between inbred strains

The data were not published in detail at the time. We shall present that bearing on the alleles S, s in condensed form. Twenty three strains were started in 1906 from single pairs by Mr. G. M. ROMMEL of the U. S.

TABLE 1

Distributions of 4 inbred strains of guinea pigs, 1916–22 in percentages. Grade O means solid color: X means a trace of white. The grades are at 5% intervals (1=2.5 to 7.5%). W (black eyed white) is distinguished from 20 (a trace of color). The median percentages of white are given separately for males and females in the last two columns.

STRAIN	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	O	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂	♀
34	100.0	0	0	0	0	0	0	0	0	333	0	0
39	0	35.2	32.9	14.3	7.7	6.7	2.3	0.9	0	659	13.9	24.4
35	0	0.8	4.0	10.1	19.7	29.4	27.1	8.6	0.3	1460	62.5	68.9
13	0	0	0	0.5	0.7	2.8	16.2	71.0	8.8	1278	95.0	96.4

Bureau of Animal Industry. These were maintained by exclusive brother-sister mating. The senior author took charge of the experiments in 1915 at which time 17 of the strains were still on hand. One of them (No. 34) had no white spotting (although much "silvering"). All of the others consisted wholly of tricolors but there were great differences among them in average amount of white. A drawing of the pattern of every animal was made at birth in a rubber stamp outline and the amount of white was estimated from an outline on tracing cloth divided into 20 squares. In table 1 is a condensed table of distributions in 4 strains. The distributions for 13, 35, 39, and two other strains have been published in full, separating males and females (WRIGHT 1926). Grade X means a trace of white and W, for black-eyed white, is distinguished from grade 20 with a trace of color. Strain 39 had least white of any of the spotted strains while 13 was at the opposite extreme.

During the years 1916 to 1919 many crosses were made between these and other strains (WRIGHT 1922). There were 1334 young from first crosses,

TABLE 2

The distributions of reciprocal crosses between strains 39 and 13, and of F_2. Note the similarity of F_2 to F_1 and the marked disagreement with expectation based on 25% strain 39, 50% F_1 and 25% strain 13 as indicated in the bottom line. In designating crosses in this and later tables, the female parent is given first.

CROSS	GRADE OF SPOTTING									NO.
	O	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
39×13	0	3.3	0	13.3	6.7	30.0	20.0	26.7	0	30
13×39	0	0.8	6.4	8.0	14.4	25.6	27.2	17.6	0	125
Total F_1	**0**	**1.3**	**5.2**	**9.0**	**12.9**	**26.5**	**25.8**	**19.3**	**0**	**155**
F_2	0	2.4	12.2	13.4	8.5	23.2	24.4	15.9	0	82
Expected in F_2 if 1 factor	*0*	*9.5*	*10.8*	*8.2*	*8.6*	*15.6*	*17.5*	*27.6*	*2.2*	

981 from crosses of F_1 to a third strain, 692 F_2's from two strains and 617 F_2's combining 4 grandparental strains. Many more were born in selection experiments from these crosses. In all of these cases, spotted by spotted produced only spotted. We shall cite data from the cross between spotted strains at opposite extremes, (13×39) in the same condensed form as above (table 2).

Reciprocal F_1's are intermediate and F_2 is also intermediate and only slightly more variable than F_1. It is impossible to find in F_2 25 per cent segregants with the distribution of strain 39 or 25 per cent like strain 13. The *minimum* number of segregating factors may be estimated at 4 or 5. No upper limit to the possible number can be set. It may be added that while males and females showed the usual slight sex difference there were no indications of sex linkage.

Let us now turn to the crosses between the single self-colored strain (No. 34) and spotted strains, all with much white. The latter will be treated collectively (table 3).

TABLE 3

The distributions of reciprocal crosses between strain 34 and miscellaneous spotted strains, of F_2, and of backcrosses of self or near self segregants to the spotted strains.

CROSS	GRADE OF SPOTTING									NO.		
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		0–5	6–W
34×spot	12.2	75.5	12.2	0	0	0	0	0	0	49	100	0
spot×34	11.1	77.8	11.1	0	0	0	0	0	0	18	100	0
Total F_1	**11.9**	**76.1**	**11.9**	**0**	**0**	**0**	**0**	**0**	**0**	**67**	**100**	**0**
F_2	9.4	53.1	9.4	12.5	0	6.2	9.4	0	0	32	71.9	28.1
1st Backcross	6.3	36.6	7.1	7.5	13.0	5.5	9.1	13.4	1.6	254	50.0	50.0
2–4 Backcross	8.6	31.9	6.9	6.9	7.7	11.2	9.5	16.4	0.9	116	47.4	52.6
Total Backcross	**7.0**	**35.1**	**7.0**	**7.3**	**11.4**	**7.3**	**9.2**	**14.3**	**1.4**	**370**	**49.1**	**50.9**

Complete dominance of self color is shown in only 12 per cent of the F_1 individuals but the average amount of white in F_1 is low, being always less than 27 per cent. In F_2, 28 per cent of the young exceed F_1, several being of high grade. There is a strong suggestion here of segregation of an incompletely recessive factor for high grade spotting. This is confirmed by the backcross of F_1 to inbred spotted. Exactly 50 per cent are above the limits of F_1. A second backcross of near self from the above matings to inbred spotted practically repeated the result from the first backcross. The same was true of small 3rd and 4th backcross generations. The young from these were derived only $\frac{1}{8}$ to 1/32 from the self strain but show no tendency to dilution of the heredity transmitted from the latter.

Non-genetic variability

The existence of the alleles *S*, *s* was first confidently asserted in a later paper (WRIGHT 1920) on the basis of a new experiment which had then reached the 7th backcross generation of self to inbred spotted. This experiment, ultimately carried to the 11th backcross generation, will be discussed later. The 1920 paper dealt primarily with an analysis of the variability within closely inbred strains. It was shown that in a typical strain (No. 35) descended at that time from a single mating in the 7th generation of brother-sister mating, there was variation from a trace of white to self-white but that this enormous variability (standard deviation about 22 per cent of the area of the coat) was almost wholly non-genetic. There was a correlation of only +.014 between parent and offspring (using a transformed scale designed to counteract the damping of variation in the neighborhoods of 0 and 100 per cent white). It was further shown that most of this non-genetic variability was of such a sort that environmental factors common to littermates played very little role. The correlation between littermates was only +.069.

While the intrastrain differences were thus proved to be non-genetic, the differences between strains are of course genetic. Both should be present in a random bred stock and this proved to be the case. The stock from which the inbred strains were derived had been maintained without even second cousin mating. In this stock the correlation between parent and offspring at this time was +.211 interpreted as indicating that 42 per cent (=2×.211) of the variance is genetic (leaving 58 per cent as non-genetic) on the assumption of no dominance or epistasis. Confirming this was the observation that the actual variance in the inbred stock was 57 per cent of that in the random bred stock on the transformed scale.

More extensive data on this question have been reported briefly in a later paper (WRIGHT 1926) together with data which indicate that the most important of the non-genetic factors common to littermates is the age of the mother. We shall discuss these and other data on the roles of heredity and environment in a later section.

There is a remarkable contrast between the situation in the guinea pig and that in the rat. In CASTLE'S (1916) selection experiments with hooded rats, the standard deviation among the offspring of the later generations averaged only about 3 per cent of the range from solid color to solid white. Yet progress by selection was possible up to the end, demonstrating the presence of considerable genetic variability. The correlation between mid parent and offspring (10th to 16th generations) averaged about +.25 and +.26 in the minus and plus series respectively. Thus a standard devia-

tion of 3 per cent in an unfixed strain of rats is to be compared with one of about 20 per cent in a stock of guinea pigs in which all animals have been shown to have almost identical genetic constitutions. The same contrast is also doubtless indicated by the difference in regularity and symmetry. The patterns of hooded rats form a practically linear series of grades with a high degree of symmetry. In guinea pigs nearly every individual has its own characteristic pattern and there is usually asymmetry. Why the same character should behave so differently in different rodents is not known. The fact of an enormous amount of non-genetic variability in the guinea pig must be taken into account in any discussion of the genetics of white spotting in this animal. The phenotypes of single individuals are practically worthless here as indicators of genotypes.

Eleven generations of back crossing

The 11-generation backcross experiment, referred to above, was reported at the 1923 meeting of the American Society of Zoologists, but only

TABLE 4

Distribution of grades of spotting in F_1, F_2 and backcrosses to spotted strains, following a cross of a self-colored male to spotted females. The backcrosses are classified according to the amount of ancestry of strains 35 (median about 65% white) and strain 13 (median at 98% white).

	GRADES OF SPOTTING									NO.
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
spot×self	26.7	70.0	0	3.3						30
F_2	10.0	45.0	10.0	10.0	10.0	5.0	10.0			20
Backcrosses to spot										
3/4 blood or more 35	7.7	44.3	7.7	17.3	7.7	1.9	9.6	3.8		52
Intermediate	1.8	33.9	8.8	5.8	10.8	11.0	14.3	12.8	0.8	398
3/4 blood or more 13	0	21.2	11.3	12.5	6.2	1.3	11.2	33.8	2.5	80

a brief abstract was published. The first cross was between a self-colored male (unrelated to strain 34 or to the other inbred strains referred to above) and tricolor females from various inbred strains. Most of the young showed a little white spotting, the most extreme being a female with 30 per cent. None showed any yellow spotting. A small F_2 generation showed 25 per cent with spotting beyond the limits of F_1. F_1 was backcrossed with tricolors of various strains and produced about 50 per cent with no more white than F_1 and about 50 per cent with larger amounts. Each of these classes was about equally divided between animals with and without yellow spotting.

Selfs or near selfs of this generation were backcrossed again to inbred tricolors producing young which were 7/8 blood tricolor by ancestry but the distribution of types was substantially as in the first backcross genera-

tion. This process was repeated through 11 backcross generations, producing at length animals which were 99.98 per cent of tricolor ancestry but the results remained the same.

The tricolor strains used varied from ones which averaged about 65 per cent white (No. 35) to ones with over 95 per cent (No. 13). In table 4 all backcross generations are combined, but a distinction is made between those which were $\frac{3}{4}$ blood or more of strain 35, those which were $\frac{3}{4}$ blood or more of strain 13 and those which came of intermediate tricolor ancestry.

Obviously the line between *Ss* and *ss* must be drawn at different points, depending on the tricolor strain used. In table 5, the line was drawn above

TABLE 5

Persistence of genes S and E through 11 generations of backcrossing of self (or near self) segregants ($SsEe^p$) to inbred tricolor stocks (sse^pe^p). Criterion for separating Ss and ss as described in text.

NO. OF BACK-CROSSES	AMOUNT OF SELF ANCESTRY	LITTLE OR NO WHITE		STRONGLY SPOTTED		TOTAL	
		NO YELLOW $SsEe^p$	TORTOISE Sse^pe^p	NO YELLOW $ssEe^p$	TRICOLOR sse^pe^p	*Ss*	*ss*
1	1/4	25	26	29	18	51	47
2	1/8	19	22	14	16	41	30
3	1/16	12	6	5	6	18	11
4	1/32	12	10	4	6	22	10
5	1/64	6	8	20	14	14	34
6	1/128	6	7	6	7	13	13
7	1/256	7	7	9	7	14	16
8	1/512	13	10	17	18	23	35
9	1/1024	15	14	18	9	29	27
10	1/2048	19	14	21	13	33	34
11	1/4096	5	4	4	2	9	6
Total		**139**	**128**	**147**	**116**	**267**	**263**

grades 3 and 4 for males and females respectively of the group with $\frac{3}{4}$ blood of strain 35, above 7 and 8 for the males and females respectively of the intermediate group, and above 11 and 12 for the males and females of those with $\frac{3}{4}$ blood of strain 13. These were the points which most nearly divided these groups on a 1:1 basis. It is of course recognized that this is somewhat arbitrary and that there was probably real overlapping in each case.

While no great stress can be put on the approximate equality of the groups with low and high grade spotting, the persistence of a bimodal distribution through 11 generations of backcrossing of selected low grades to pure tricolors can only be interpreted as evidence of transmission of an undilutable unit (*S*). The persistence of a group with no yellow spotting through the 11 backcrosses of course indicates transmission of a second

unit (E) and the ratios indicate random assortment relative to S,s. The deficiency of tricolors is undoubtedly due to the failure of yellow spotting to appear in some of constitution sse^pe^p which were nearly or wholly white. The fact that the white spotting factor s behaves as a modifier of the tortoiseshell pattern was obvious in these data. In Sse^pe^p with little white, the yellow was usually restricted to scattered hairs or an irregular brindle. In sse^pe^p there was a more or less complete segregation of black and yellow into distinct spots on the white ground. These effects have also been noted by ILJIN (1928). The yellow spotting factor on the other hand had no apparent modifying effect on the white pattern.

Other interpretations of spotting

PICTET has also reported on extensive experiments with white spotting in guinea pigs. From his descriptions and illustrations, it seems clear that the patterns in his stocks are similar to those with which we have worked. His conclusions, however, are very different. He finds four major pairs of alleles, two affecting head only and two the trunk only, no one of which can be identified directly with our single major pair S, s.

In a paper (1925) dealing with white on the head he distinguishes self color, frontal white (self except for a median streak) and lateral white (white on cheeks as well as nose). Pure selfs by albinos gave 78 self to 82 frontal (approximately 1:1). Crosses between these F_1 types gave 54 self: 54 frontal: 36 lateral (exact 3:3:2) apart from albinos. He assumes two pairs of alleles with interactions as below.

	FF, *Ff*	*ff*
UU, *Up*	Frontal	Self
pp	Lateral	Lateral

It is assumed that the selfs were of type *UUff* and that all of the albinos happened to be *ppFf* (the albino factor being correctly interpreted as independent of spotting). Results from later generations are interpreted as in harmony with this hypothesis.

Later papers (1930, 1931) analyze spotting of the trunk. While it is stated that in most cases the extent of white is similar on head and trunk, it is held that these regions are subject to independent systems of genes because the correlation is not complete. In particular it is noted that animals with "generalized" white on the trunk may have a self colored head while one with a self colored trunk may have any amount of white on the head. Thus while the symbols U and p are used for genes affecting the trunk in these papers, they are apparently not considered to be related to the head factors assigned these same symbols in the earlier paper.

From the 1931 account, 5 original matings of self by spotted gave 42 spotted in F_1, and 52 spotted to 19 self in F_2, suggesting a dominant gene

for spotting. On the other hand, 5 other matings of self by spotted gave 49 self in F_1, and 50 self to 19 spotted in F_2, suggestive of recessive spotting. The spotting in these two cases differed in character. The white in the recessive spotting was limited to the feet and small spots on the nape of the neck and in the sternal and perianal regions (apart from the head). This is called localized spotting. In the dominant type, the white was more or less extended over the body, ranging from narrow streaks across the back or along the belly to the completely white type (with black eyes). This is called generalized spotting.

The descendants of the above crosses were interbred. The total ratios agree with expectation from formulae assigned to parents according to the following scheme of combination effects.

	Pp, *Pp*	*pp*
UU, *Uu*	Generalized	Self
uu	Localized	Localized

($\chi^2 = 1.2$, n = 12 probability more than .999 of worse fit from random sampling.)

IBSEN (1932) makes the following statement in regard to white spotting. "White spotting (*s*) is quite variable in its expression in the guinea pig. There are probably a number of modifiers concerned and in addition there seems to be some variation that is entirely somatic. The dominant modifier *Fa* (face) causes the white spotting to be restricted entirely to the face, while its allelomorph, *fa*, permits white to appear in other parts of the body as well. Evidence has been accumulating which seems to indicate that there is a modifier of *Fa*, thus being a modifier of a modifier. This factor, *Na*, (narrow) causes the white face to be narrow while its allelomorph, *na*, permits it to widen. *Na* seems to be completely dominant to *na*. There are other types of white spotting in guinea pigs, such as white belt and white rump and it is even possible by selection to produce animals that are entirely white. None of these, however, can be readily fixed by selection thus lending support to the supposition that there is more than one pair of modifiers concerned in the production of each type. It would probably require much research to make a complete analysis of the modifiers affecting white spotting."

This scheme may be represented as follows. The data supporting it have not been published.

S – – –	Self
ss Fa – *Na* –	Narrow facial streak only
ss Fa – *na na*	Extensive facial white
ss fafa –	White on body as well as face

This scheme resembles that presented by WRIGHT in assigning one main factor to spotting but differs in treating this as completely recessive.

The greatest difference is with respect to non-genetic variability. IBSEN finds so little that definite formulae can be assigned certain phenotypes, while we have found so much in all inbred spotted stocks that such an assignment would be quite impossible.

New crosses between inbred strains

Thus since 1932 there have been three widely different interpretations of the genetics of white spotting of the guinea pig. It seemed desirable to make a new series of tests. The following experiments involve only three closely inbred strains.

Strain D has been closely inbred since 1906 first by Prof. W. E. CASTLE since 1916 by WRIGHT. It has never thrown white spotting of any sort.

Strain 2, as used here, is entirely descended from a single mating in the 15th generation of brother-sister mating (U. S. D. A strain, p. 759). It has

TABLE 6

Distributions of spotting in strains 2, 13, in reciprocal crosses between them and in F_2.

	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂	♀
Strain 2				0.2	2.0	5.8	20.8	68.2	3.0	1650	93.2	94.6
Strain 13					0.1	1.2	12.5	62.4	23.8	1688	97.0	98.6
2×13							28.6	71.4		28	94.0	93.8
13× 2						3.3	13.3	73.3	10.0	60	95.9	96.4
Total F_1						**2.3**	**18.2**	**72.7**	**6.8**	**88**	**95.2**	**95.4**
F_2					1.9		28.8	69.2		52	94.4	96.9

consisted wholly of high grade spotted (median percentage of white 93.2 in males, 94.6 in females). There has been, however, considerable variability in grade as shown in table 6. The correlation of $+.064 \pm .025$ between parent and offspring (here made without any transformation of scale, but arbitrarily treating black eyed white as a grade higher than grade 20 with a trace of color) shows that this variability is largely non-genetic (see table 16).

Strain 13 as used here traces to a single mating in the 18th generation of brother-sister mating. Two substrains are distinguished, 13 and 13E, the former but not the latter from continued brother-sister mating. There is, however, no recognizable difference between them in amount of white, which is the greatest of any inbred strain we have had. The median grades are 97.0 in males, and 98.6 in females. As with strain 2, the absence of appreciable correlations between parent and offspring (table 16) indicates that practically all variability is non-genetic.

Crosses have been made between strains 2 and 13 which indicate that

TABLE 7

Crosses involving only strain 2 (ss with median about 94 per cent white, table 6) with strain D (SS, always self colored).

MATING	AMOUNT OF D ANCESTRY	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO.	MEDIAN IN ♂♂	MEDIAN IN ♀♀
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W			
2×D	1/2 D	*ss*×*SS*	91.5	8.5								59		
F_1×D	3/4 D	*Ss*×*SS*	95.7	4.3								207		
1 BC×D	7/8 D	(*Ss*, *SS*)×*SS*	99.0	1.0								305		
2 BC×D	15/16 D	(*Ss*, *SS*)×*SS*	100.0	0.0								182		
3 BC×D	31/32 D													
F_1×F_1	1/2 D	*Ss*×*Ss*	46.2	23.1		3.8		23.1	3.8			26		
2 BC×2 BC	7/8 D	*SS*×*S*-	100.0									21		
		Ss×*Ss*	71.0	22.6	6.4							31	6.8	5.0
2 BC×7/8	7/8 D	*Ss*×*ss*	45.9	45.9	5.4		2.7					37		
7/8×7/8	7/8 D	*ss*×*ss*	0	69.7	20.7	6.9	0.7	1.4	0.7			145	6.5	12.1
7/8×2	7/16 D	*ss*×*ss*			14.1	18.7	26.6	20.3	12.5	7.8		64	47.5	55.8
(7/8×2)×7/8	21/32 D	*ss*×*ss*		45.4	18.2	27.3	9.1					11	12.5	30.0

they are closely similar genetically. The distributions of F_1 and F_2 are shown in table 6. The medians (95.2 and 94.4 per cent for F_1 and F_2 males respectively and 95.4 and 96.9 per cent for F_1 and F_2 females respectively) give no indication of any complementary effect and the doubtful increase of variability in F_2 gives indication of only minor differences.

Strain 2 (*ss*) had been mated with strain D (*SS*) for another purpose and repeated backcrosses had been made to D (WRIGHT 1935a). F_1 (table 7) consisted of 59 animals (all *Ss*) of which 54 were completely self while 5 had a trace of white (one or both hind feet). This result from a cross to a strain as white as 2 indicates that D must have a rather exceptional stock of modifiers which repress white. Among 207 backcross animals (about half *Ss*, half *SS*) 198 showed no white while 9 had traces consisting in most cases of a few white hairs between the ears. Among 305 of the second generation of backcrossing to D (about 25 per cent *Ss*, 75 per cent *SS*) only 3 showed a few white hairs. 182 animals which were 15/16 or 31/32 blood of strain D were entirely self although it is probable that some were still heterozygous. Evidently *S* is almost invariably fully dominant over *s* where there is a preponderance of modifiers from strain D.

Some of the 7/8 blood animals (all self colored) were tested by mating with strain 13. Eight of these produced only self or low grade spotted among 83 young and were considered to be *SS*. The distribution of grades is shown in table 8. Two males and two females gave some high grade spotted, presumably *ss*, demonstrating the tested self parent to be *Ss*.

These tested heterozygotes were now mated *inter se* in order to extract a spotted line deriving its spotting from strain 2 but 7/8 of its modifiers from strain D (table 7). Their young consisted of 22 fully self and 9 low grade spotted. Matings of tested heterozygotes with these spotted segregants produced 17 fully self and 20 low grade spotted. Matings among these spotted animals and their descendants have produced 145 all spotted and, with few exceptions, of low grade. These results indicate complete recessiveness of spotting in animals which are 7/8 blood of strain D. Most of them were of what PICTET calls the localized type (where spotted at all on the trunk). The results are thus close to those which he has reported for his recessive spotting factor except that white on the head (few white hairs to extensive white on nose and cheeks) nearly always accompanied the localized spotting of the trunk, indicating that only a single main factor was involved.

These animals have necessarily derived their white spotting from strain 2 (with generalized white). The great difference in average grade of white must therefore be due to independent modifiers. Matings back to strain 2 have produced 64 young with median grade about half way between the parents.

TABLE 8

Crosses between strain 13 and animals which were 7/8 blood strain D, 1/8 blood strain 2.

	BLOOD OF 13	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO.	MEDIAN % W			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
7/8 D×13	1/2	*SS*×*ss*	19.3	72.3	6.0	2.4						83	0.9	1.7		
		Ss×*ss*		46.2		7.7	7.7	7.7	23.1	7.7		13			42.5	72.5
		ss×*ss*			20.0	20.0	40.0	20.0				5				

TABLE 9

Repeated backcrossing of near self segregants to strain 13, illustrating persistence of gene S in spite of continued attempts at dilution. Reciprocal backcrosses are combined in this table.

Ss×*ss*	BLOOD OF 13	GRADES OF *Ss* PARENTS	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
1/2 (13)×13	3/4	All X	—	43.6	5.1	2.6	2.6	6.4	11.5	24.4	3.8	78	1.6	7.2	87.8	95.6
3/4 (13)×13	7/8	X–6	—	17.9	14.9	14.9	—	6.0	9.0	25.4	11.9	67	16.2	24.2	93.1	98.2
7/8 (13)×13	15/16	X–6	—	18.9	16.8	6.3	3.2	2.1	12.6	38.9	1.1	95	14.1	17.0	93.0	93.1
15/16(13)×13	31/32	3	—	16.7	25.0	8.3	—	—	8.3	25.0	16.7	12				
31/32(13)×13	63/64	2–6	—	5.9	11.8	21.6	2.0	7.8	11.8	29.4	9.8	51	16.7	31.2	96.2	96.7
63/64(13)×13	127/128	8–9	—	22.7	9.1	4.5	—	9.0	18.2	18.2	18.2	22				

TABLE 10

Tests of 15/16 blood segregants from backcross matings to strain 13 and of their inbred descendants. Low grades (Ss) produce a ratio which can be interpreted as 1 SS: 2 Ss: 1 ss while high grades (ss) breed almost like strain 13.

SOURCE	MOTHER CONSTITUTION AND GRADE	FATHER CONSTITUTION AND GRADE	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
15/16 backcross	*ss* (16–20)	*ss* (16–20)					0.8	2.3	29.0	56.5	11.5	131			92.5	96.5
Descendants	*ss* (14–20)	*ss* (17–19)						8.2	20.4	53.1	18.4	49			93.9	95.0
15/16 backcross	*Ss* (1–9)	*Ss* (X–8)	12.5	31.6	11.8	10.3	2.2	2.9	11.0	13.2	4.4	136	12.0	28.5	88.4	97.7
Descendants	*Ss* (1)	*Ss* (X)	22.2	50.0	11.1			5.6		5.6	5.6	18				
	S- (0–1)	*SS* (0–X)	26.3	69.7	3.0		1.0					99				
	SS (0–1)	*ss* (15–20)		50.0	30.0	10.0	10.0					10				
15/16 backcross	*ss* (16)	*Ss* (9)			20.0			20.0		60.0		5				
Descendants	*ss* (19)	*Ss* (14)		30.0				10.0	40.0	20.0		10				

We will now turn to the descendants of the test crosses to strain 13 (table 9). Animals with only a trace of white were selected and backcrossed to 13 (*ss*). The process was repeated for several generations. No fully self-colored animals have appeared in these backcrosses. A markedly bimodal distribution of grades of spotting has, however, persisted throughout. In those 7/8 blood or more of strain 13, the median of the lower group (*Ss*) was, however, higher than that of the extracted spotted (*ss*) with 7/8 blood of strain D, while the median of the higher group is nearly the same as that of pure 13. As in the previously reported experiments of this type, there can be little doubt of the segregation of a single major unit factor. As a critical test, however, matings were made within each of the groups of supposed segregants (table 10). The matings between high grade spotted (grades 16 to 20) gave an array almost like that of pure 13. These were clearly *ss*×*ss*. Supposed heterozygous segregants (grades X to 9) of these same generations were also mated *inter se*. The progeny were utterly different. Of the 136 young, 17 were fully self and 24 more had only a trace of white. About 66 per cent had more color in the coat than the most colored of the young from the high grade parents. This result leaves no doubt of the conclusion that there is here segregation of a single spotting factor showing incomplete dominance over self. The results from the crosses between 2 and 13 show that it is the same spotting factor that was completely recessive where there was 7/8 blood of strain D.

As noted 17 of the 136 young from *Ss*×*Ss* were recorded as self. This is only 12.5 per cent or half the proportion of *SS* expected, indicating that a small amount of white may be brought out by the "modifiers" of strain 13 even in the absence of the main spotting factor. Unfortunately strain 13 is silvered as well as spotted. The above 17 animals showed more or less silvering but no white in the regions (nose, forehead and feet) where piebald white is most likely to appear. Those recorded as having a trace or more of piebald white all showed white in one or more of these regions.

Tests of grading

It seemed desirable to test the reliability of the grades. As noted, these were based on drawings made in rubber stamp outline. Most of the drawings were made by the senior author at the time of recording births. The estimates of grades were made by the junior author, using an outline on tracing cloth, divided into squares. In 306 cases (all from backcrosses of *Ss* to *ss* of strain 13 and including all grades from a trace of white (x) to black eyed white (w) estimates were made later by the senior author without knowledge of Mr. CHASE'S grades. This comparison tests the accuracy of grading but not, of course, of the drawings. The statistical

analysis of the two series of grades (not using Shepard's correction) gave the following results.

	Mean	*Standard Deviation*	*Correlation*
Chase	11.41	7.96	.9978 ± .0003
Wright	11.36	7.93	

There is no indication of any differential systematic errors and random errors are of negligible importance. Only about 0.2 per cent of the variance is determined by random errors.

In another test, Mr. CHASE made wholly independent records of the patterns of 119 miscellaneous animals, distributed rather uniformly from grade x to 20, but including no black eyed whites. The two sets of drawings were also graded independently.

	Mean	*Standard Deviation*	*Correlation*
Chase	9.99	6.53	.989 ± .002
Wright	10.11	6.52	

There can be no important differential systematic errors either in drawing or grading. Random errors are naturally considerably increased but yet account for only one per cent of the variance. As the standard deviation in this population was nearly twice that of an isogenic stock with mean near grade 10, it may be estimated that about 3 or 4 per cent of the non-genetic variance may be attributed to the combined errors of drawing and grading.

Aberrant results

Results which at first seemed rather aberrant have been obtained from crosses between two stocks which are not related to any of the inbred strains of the preceding experiments. Strain A was built up to be dominant in a large number of genes. It consists typically of self agoutis (*SEACPFB*) with smooth fur except on hind toes (*RM*). Strain B was made up to be recessive in most of these respects. It consists of smooth-furred pink-eyed, dilute brown-yellow-white tricolors (*se^{p}ac^{k}pFbrm*). The results of a large backcross generation have been described in a previous paper (1928). We are indebted to Dr. STRANDSKOV for use of recent data from these stocks and their crosses.

The earlier records of strain A naturally revealed heterozygosis in many respects including *S,s*. Since 1926, however, only low grade spotting has appeared. The complete records, 1926–1934 inclusive, are given in condensed form in table 11. The records for strain B are given for the years 1924–25 as reported previously and separately for the period 1926–34. The previous paper reported F_1 (B×A) only from 3 males of strain A, demonstrated to be homozygous *SS*. These records, it turns out, give a

far from adequate picture of the situation. The total records for F_1 (B×A) and (A×B) 1926–1934, are reported here. The backcross data (F_1×B) are merely repeated (in condensed form) from the previous paper.

The first question raised by these data is whether *ss* can ever be completely self-colored. No such case was present in the data described earlier in this paper although the median grade of white was very low in certain strains (39,7/8D) and occasional animals had only a few white hairs. The records of 851 animals from strain B from 1926 to 1934 agree in this respect, but in the earlier records of B, 4 animals out of 454 (about 1 per

TABLE 11

White spotting in strains A, B and crosses. All A's are believed to be SS in spite of some low grade spotting (up to grade 2), all B's ss in spite of 4 selfs (grade 0). Note segregation of self in F_2 from strongly spotted F_1's.

SOURCE	REMARKS	GRADE OF SPOTTING								NO.
		0	X-2	3–5	6–8	9–11	12–14	15–17	18–20	W	
A	20 Matings (0×0)	100.0									96
	17 Matings (0×0)	86.2	13.8								217
	7 Matings (X×0)	73.2	26.8								41
	Total	**88.4**	**11.6**								**354**
B	1924–25	0.9	5.7	9.0	7.7	13.2	20.7	18.3	24.4	—	454
	1926–34	0	5.9	9.4	10.9	15.9	20.0	17.0	20.7	0.2	851
F_1	A×B low	36.9	63.1								160
	A×B high		87.5	12.5							16
	B×A low	29.8	70.2								242
	B×A high	16.7	69.8	5.2	3.1	2.9	1.2	1.0	0.2		420
	Total	**24.0**	**69.0**	**2.9**	**1.6**	**1.4**	**0.6**	**0.5**	**0.1**		838
F_1×B		9.8	37.8	12.4	8.9	6.6	9.4	8.7	6.4	0.0	437
F_2	From selected F_1's (grades 9–12)	17.2	41.4	6.9	3.4	10.3	6.9	3.4	10.3	0.0	29

cent) were recorded as completely self colored although from homozygous spotted parents. Unfortunately this question was not definitely in mind at the time of record and there may have been less care in looking for traces of white than in later experiments. We think it probable that plus modifiers may occasionally bring about complete absence of white spotting in *ss* but hesitate to affirm this positively until a case has been examined with this point in mind and has been tested genetically.

Other questions are raised by the traces of white in strain A itself and the very high grade white spotting (up to 95 per cent white) found in some of F_1 (B×A). In strain A only self-colored males have been used since 1926, but in 7 cases females were used which had traces of white. Six of

these females produced at least one offspring each, with traces of white. The other had only 2 young. The total ratio was 30 self to 11 with traces of white. The remaining matings of strain A (all self by self) may be divided into two groups: 20 matings with 96 young all self-colored, and 17 matings with at least 1 white-marked offspring each and a total ratio of 187 self to 30 with white. Two of the latter had white belts across the shoulders (grade 2).

If white spotting were due to a simple recessive in this stock, we should expect more than 50 per cent from matings classified as $Ss \times ss$ by the occurrence of at least one spotted young one, but only 27 per cent were observed. We should expect more than 25 percent from matings similarly classified as $Ss \times Ss$, but only 14 per cent were observed. This hypothesis is ruled out unless *ss* is frequently self-colored, which is contrary to all previous experience. The traces of white in strain A are clearly not due to *ss*.

The next possibility is that these white-marked animals are *Ss*. If this is true, many selfs in the strain must also be *Ss*, so many that a considerable number of reasonably high grade spotted (*ss*) should have appeared. But this has not been the case since 1926. More evidence on this hypothesis is provided by the crosses with strain B. Table 11 distinguishes matings which produced at least one offspring above grade 2 (12.5 per cent white) from those which did not and in which the parent from strain A was certainly *SS*. In the former there may be a suspicion that it was *Ss*. However, the young above grade 2 from these matings constitute only 13.5 per cent of the total, a proportion so low as to practically rule out this hypothesis. It may be noted that the low ratio applies not only to the total but to every individual mating. The most extreme spotted (over 50 per cent white) came from 9 males of strain A. These produced the following ratios (making the cleavage at 12.5 per cent): 20:10, 28:7, 22:4, 31:3, 16:1, 25:3, 25:5, 6:1 and 2:1. None of these is in harmony with the mating formula $Ss \times ss$. Thus it is strongly indicated that all of strain A, self and white-marked alike, were *SS* and that these strongly marked F_1's from $(B \times A)$ reaching 95 per cent white in one case, as well as the selfs (24 per cent of the total) were all *Ss*.[1]

This is confirmed by a number of tests. Matings between rather strongly marked F_1's (grades 9 to 12) produced the F_2 population in the bottom line of table 11. The appearance of as many as 5 self and 12 with only a

[1] These crosses were in part from an experiment in which Dr. STRANDSKOV was testing the effect of X-radiation of males and many of the highest grade F_1's were from treated males. Fourteen males produced 12 above grade 2 out of 163 young before raying and 29 above grade 2 out of 191 after raying, a difference with a probability of being exceeded by random sampling of only .02. There is some suggestion here of a modifying effect.

trace of white out of 29 young make it certain that the F_1 parents were *Ss* (not *ss*). In other cases F_1's of various grades were mated with strain 13 (the whitest of the inbred strains) (table 12). There is no appreciable difference in the results from low grade and high grade F_1's and both agree with expectation from *Ss*×*ss* and not at all with that from *ss*×*ss*.

TABLE 12

Tests of F_1 (A×B) of widely different grades by mating to the most extreme strain No. 13. Both groups of F_1's breed like Ss.

	GRADE OF SPOTTING									NO.
	o	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
F_1 (2–6)×strain 13	o	32.0	10.0	6.0	4.0	8.0	16.0	24.0	—	50
F_1 (13–17)×strain 13	o	20.4	12.2	4.1	10.2	10.2	14.3	28.6	—	49

These results from (B×A) may be compared with those in table 13 from the mating (B×D). It will be recalled that strain D was wholly self (*SS*) and gave results in crosses with the high grade spotted of strain 2 which indicated an expectional array of modifiers which reduce white.

TABLE 13

Crosses of strains B and D.

		GRADE OF SPOTTING									NO.
		o	X	1–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
F_1(B×D)	*ss*×*SS*	77.9	22.1									68
F_2(B×D)	*Ss*×*Ss*	57.3	24.2	4.4	5.2	3.9	2.3	1.0	1.3	0.3		384

The results from (B×D) are quite as expected. The white-suppressing modifiers of strain D have reduced *Ss* to a distribution similar to that of *SS* of strain A, and the *ss* segregants in F_2 must have a distribution much like that of *Ss* of F_1 (B×A).

Analysis of variability

Having established that there is just one major pair of alleles affecting white spotting in a number of diverse stocks, it is desirable to return to the evaluation of the roles of heredity and environment in determining the variation within a spotted stock. In doing this it is desirable to allow for the obvious damping of variability at both extremes of the range from self color to self white. An approximate method of doing this has been discussed in previous papers (WRIGHT 1920, 1926). The assumption that the pigmentation tendencies of the various areas of the coat are distributed normally leads to the transformation $X' = \text{prf}^{-1}(X - .50)$ where X is the proportion of color in the coat and prf^{-1} is the inverse probability function.

For the purpose of calculating the correlations, the grades were grouped in pairs and a transformed grade was found for the mid point of each (table 14). Table 15 shows the correlations between parents, between parent-offspring by sex and between littermates also by sex in a random bred stock and in a portion of strain 35 derived from a single mating in the 12th generation of brother-sister mating, using the above transformation of scale. The records for these two stocks were made during the same period of years (1916 to 1924) and are thus strictly comparable. The same set of correlations but without the transformation of scale has been found for a more recent branch (1926–1934) of strain 35, tracing to a single mat-

TABLE 14

Transformed grades, based on the inverse probability function of the midgrades and used in calculating standard deviations and correlation coefficients.

GRADE	MIDGRADE PERCENTAGE (X)	TRANSFORMED GRADE X' $\left(X' = \text{prf}^{-1}\frac{(X-50)}{100}\right)$
X– 2	6.25	−1.534
3– 4	17.5	− .935
5– 6	27.5	− .598
7– 8	37.5	− .319
9–10	47.5	− .063
11–12	57.5	+ .189
13–14	67.5	+ .454
15–16	77.5	+ .755
17–18	87.5	+1.150
19–20	96.25	+1.780

ing in the 22nd generation (35 D) (table 15) and for strain 2 and the two branches of strain 13 described earlier in this paper (table 16). In the case of 35 D, the correlations among brothers and sisters which were not littermates were also found. The correlations between brothers and between sisters were calculated from symmetrical tables, each pair being entered twice.

In no case was there a significant amount of assortative mating. There are clearly significant correlations between parent and offspring in the random bred stock (average +.191) but none that are significant among the inbred strains (grand average +.016). The littermate correlations (+.282) are larger than the parent-offspring correlations (+.191) in the random bred stock; and though small are clearly significant among the inbred strains (grand average +.079). These results can only be due to environmental factors common to littermates. This interpretation is confirmed by the absence of positive correlation (−.048) between siblings which were not littermates in the inbred strain 35 D.

For analysis of the variability we can use only the data based on the transformed scale. If h^2 represents the portion of the variance due to heredity, e^2 that due to environment common to littermates and d^2 that due to environment not common to littermates; the correlation between parent and offspring should equal $\frac{1}{2}h^2$ if the effects of all genes combine additively (no dominance or epistasis) but should be somewhat less if these conditions are not met (WRIGHT 1920). Under the same conditions the

TABLE 15

Parent-offspring and fraternal correlations in a random bred stock, and in the inbred strain No. 35 at two periods. Transformed grades used in case of random breds and the earlier data from strain 35. Untransformed grades used in 35 D.

		RANDOM BRED STOCK		STRAIN 35 (1916–24)		STRAIN 35 D (1926–34)	
		NO.	r	NO.	r	NO.	r
Father-Mother		143	+.060	140	+.064	72	−.047
Parent-offspring	♂-♂	973	+.244	738	+.015	200	−.068
	♂-♀	929	+.187	688	+.074	190	+.149
	♀-♂	1014	+.217	738	+.013	196	+.052
	♀-♀	965	+.116	688	−.004	185	−.031
	Average	**3881**	**+.191**	**2852**	**+.024**	**771**	**+.026**
Littermates	♂-♂	537	+.355	340	+.128	91	+.024
	♂-♀	1050	+.288	722	+.089	180	+.109
	♀-♀	493	+.190	305	+.107	89	+.218
	Average	**2080**	**+.282**	**1367**	**+.103**	**360**	**+.115**
Sibs not littermates	♂-♂					335	−.061
	♂-♀					509	−.049
	♀-♀					202	−.021
	Average					**1046**	**−.048**

correlation between littermates would be $\frac{1}{2}h^2+e^2$. The assumption of complete dominance and equal frequencies of alleles reduces the parent-offspring correlation to $1/3h^2$ and that between littermates to $5/12h^2+e^2$. In all cases, dominance reduces the genetic component of the fraternal correlation just half as much as it does the parent-offspring correlation (PEARSON 1909; WEINBERG 1910; FISHER 1918). Any non-additive effects among genes which are not alleles (epistasis) also reduces the correlations between relatives (WRIGHT 1935).

The average standard deviation of males and females in the random bred stock was .757, that in strain 35 was .583 (transformed scale). The

variance of strain 35 is thus 59 per cent $\left(\frac{.583^2}{.757^2}\right)$ of that of the random bred stock indicating that about 41 per cent of the latter variance was due to heredity, and had been lost after long continued inbreeding of strain 35. The correlation between parent and offspring in the random-bred stock (+.191) indicates 38 per cent (=2×.19) of the variance as due to additive gene effects. This differs so little from the 41 per cent estimated from the actual variances that little complication from non-additive gene

TABLE 16

Parent-offspring and fraternal correlations in 3 inbred strains, characterized by very large amounts of white. Untransformed grades.

	STRAIN 2 (1926–34)		STRAIN 13 (1926–34)		STRAIN 13 E (1926–34)		TOTAL	
	NO.	r	NO.	r	NO.	r	NO.	r
Father-Mother	94	+.019	86	+.220	105	+.027	285	+.089
Parent-offspring ♂-♂	402	+.035	486	+.007	368	−.078	1256	−.012
♂-♀	437	+.149	436	+.036	375	−.132	1248	+.018
♀-♂	408	+.044	488	+.041	366	−.019	1262	+.022
♀-♀	433	+.029	436	−.049	375	+.029	1244	+.003
Average	**1680**	**+.064**	**1846**	**+.009**	**1484**	**−.050**	**5010**	**+.008**
Littermates ♂-♂	179	+.098	250	+.096	188	+.011	617	+.071
♂-♀	372	+.074	451	+.090	358	+.005	1181	+.059
♀-♀	215	+.068	213	+.025	166	+.054	594	+.049
Average	**766**	**+.078**	**914**	**+.077**	**712**	**+.018**	**2392**	**+.060**

effects (dominance or epistasis) is indicated. It should be said, however, that another method of calculating the transformed standard deviations of the inbred and random bred strains was made by fitting the distributions by the function $\text{prf}^{-1}(\Sigma f - .50) = 1/s\,[\text{prf}^{-1}(X - .50) - a]$ where a and s are the mean and standard deviation on the transformed scale and Σf the running sum of the fractional frequencies (WRIGHT 1926). This yields 54 instead of 59 per cent as the ratio of the variances (s=.782 in random bred stock, s=.574 in strain 35). A somewhat greater role of non-additive gene effects is indicated by this method. The difference was due mainly to irregularities in the distribution of the random bred animals.

The females exceeded the males in amount of white by .241 on the transformed scale in the control stock. This is 31.3 per cent of the standard deviation of the total population (.768). In strain 35, the difference was

.209 or 35.2 per cent of the standard deviation of the total population (.593). The proportion of the total variance due to sex is given by the ratio of the square of the half difference to the total variance, or one fourth the square of the above fractions. It appears that 2.5 per cent of the variance in the random bred stock and 3.1 per cent in strain 35 was due to sex. The analysis into genetic and nongenetic portions applies of course to the 98–97 per cent not due to sex.

The littermate correlation in strain 35 (+.103) can be taken as indicating the portion of the variance (within each sex) which is due to environment common to littermates. This is on the assumption that the

TABLE 17

Mean percentages of white in males and females of strain 35, at two periods, in relation to age of mother.

AGE OF MOTHER (MONTHS)	STRAIN 35 (1916–24)				STRAIN 35 D (1926–34)			
	MALES		FEMALES		MALES		FEMALES	
	NO.	AV.	NO.	AV.	NO.	AV.	NO.	AV.
3– 5	182	56.3	153	60.5	25	56.0	31	65.0
6– 8	195	59.5	187	67.6	45	67.8	41	70.2
9–11	152	60.6	160	66.5	40	71.7	21	78.1
12–14	150	61.3	124	70.6	33	69.8	30	76.3
15–20	174	63.2	149	69.6	27	61.9	34	74.7
21–46	138	66.9	144	73.3	23	78.2	25	77.6
Total	**991**	**61.1**	**917**	**67.8**	**193**	**67.8**	**182**	**73.1**

parent-offspring correlation (+.024) can be ignored. If the latter is accepted, the estimate is reduced to +.079. The figures from the other inbred strains confirm the existence of an effect of the order of these figures as does the difference (+.091) between littermate and parent-offspring correlations in the control stock. It is indicated that the greater portion of the variance (89.7 per cent in strain 35) is due to environmental factors so local in incidence that they do not affect littermates alike.

The only direct evidence on the nature of the environmental factors common to littermates is given by tabulations of grade of spotting in relation to age of mother. Such tabulations have been made in strains 35, 35 D, 2 and 13. In the last two cases no significant relations were found, but the highly asymmetrical distributions banked up against the limit of 100 per cent white make these unfavorable material for detecting slight differences. The results in 35 and 35 D are shown in table 17. In strain 35, there is a fairly regular increase in percentage of white with increasing age of the parents (usually littermates) at a rate of 0.68 per cent per month

TABLE 18

Joint frequencies of parent and offspring in inbred strain 35. Correlation (broad categories) +.026

PARENT	OFFSPRING GRADE OF SPOTTING X-	3-	5-	7-	9-	11-	13-	15-	17-	19-	TOTAL
19–20	—	1	5	6	8	7	22	18	10	4	81
17–18	1	7	28	22	51	68	82	73	53	14	399
15–16	6	8	24	43	38	80	97	96	57	17	466
13–14	2	7	25	35	74	90	103	121	72	14	543
11–12	6	8	22	44	59	102	105	99	77	21	543
9–10	3	7	19	24	34	60	66	58	62	10	343
7– 8	3	6	14	29	34	64	51	63	45	8	317
5– 6	—	2	6	13	22	15	28	20	16	1	123
3– 4	1	—	—	2	1	1	1	1	1	—	8
X– 2	2	—	1	2	5	5	7	3	3	1	29
Total	24	46	144	220	326	492	562	552	396	90	2852

up to 15 months of age. This factor determines about 3.6 per cent of the total variance and thus approximately half of that common to littermates. In 35 D, the results are more irregular as expected from the smaller numbers but the correlation of $+.252 \pm .069$ for females, $+.203 \pm .069$ for males give clear evidence of significance (the average $+.227 \pm .049$ is 4.6 times its standard error). From the square of this figure it appears that about 5 per cent of the variance (untransformed scale) was due to this factor. This would be slightly reduced by transformation of the scale. The regression, 0.76 percent white per month (up to 15 months) agrees reasonably well with the earlier data. It should be noted that in case (35 D) the effect is definitely one of age of mother, since most matings were intentionally

TABLE 19

Comparison of correlations obtained by the broad category method with the averages of those given in tables 15 and 16.

STRAIN	PARENT-OFFSPRING NO.	CORRELATION TABLES 15, 16	CORRELATION BROAD CAT.	LITTERMATES NO.	CORRELATION TABLES 15, 16	CORRELATION BROAD CAT.	SIBS, DIFFERENT LITTERS NO.	CORRELATION TABLE 15	CORRELATION BROAD CAT.
35	2852	+.024	+.026	1367	+.103	+.075			
35D	771	+.026	+.020	360	+.115	+.083	1046	−.048	−.045
2	1680	+.064	+.059	766	+.078	+.101			
13	1846	+.009	+.008	914	+.077	+.090			
13E	1484	−.050	−.066	712	+.018	+.005			
Av. 5 inbred strains	8633	+.016	+.012	4119	+.079	+.072			
Random bred-stock	3881	+.191	+.199	2080	+.282	+.260			

made between males and females of widely different ages (WRIGHT 1935, p. 526).

It has seemed desirable to check the parent-offspring and littermate correlations by a different method of allowing for the distortion of the scale. For this purpose the tabulations by sex were combined into single tables and correlations calculated by PEARSON'S method for broad categories. The parent-offspring distribution for strain 35 is shown in table 18; those for littermates in this stock and those in the random bred stock have been deposited with "Genetics." In the broad category method, the mean

TABLE 20

Deviations of class limits from the median in the random bred stock and in strain 35 in terms of their standard deviations assuming normality. See fig. 1.

CLASS LIMIT (% WHITE)	RANDOM BRED STOCK		STRAIN 35	
	100 q	$\frac{x}{\sigma}$	100 q	$\frac{x}{\sigma}$
0	0	$-\infty$	0	$-\infty$
12.5	1.37	−2.206	0.84	−2.391
22.5	3.46	−1.817	2.45	−1.969
32.5	7.09	−1.469	7.50	−1.439
42.5	13.17	−1.118	15.21	−1.027
52.5	21.62	− .785	26.64	− .624
62.5	32.88	− .443	43.89	− .154
72.5	47.90	− .053	63.60	+ .348
82.5	66.09	+ .415	83.95	+ .952
92.5	80.98	+ .877	96.84	+1.858
100	100.00	$+\infty$	100.00	$+\infty$

of each class on a hypothetical normal scale, is calculated by the formula $x=\frac{z_1-z_2}{q_1-q_2}$ where q_1 and q_2 are the observed tail frequencies (fractions of 1) cut off by the class limits and z_1 and z_2 are the theoretical ordinates of the unit normal curve at these points. The approximate correlation is given by the formula $r_{x_1x_2}=\frac{\Sigma x_1x_2f}{n\sigma_{x_1}{}^2\sigma_{x_2}{}^2}$. The results are given in table 19. Comparisons are made with the averages of the correlations for separate sexes from tables 15 and 16. The latter might be expected to be slightly larger because of the elimination of sex differences but the different scales prevent exact comparison. On the whole the differences are unimportant.

In this method, the scale is adjusted in each case so as to yield a corrected standard deviation of 1. Thus each distribution yields a separate scale. The transformed location of each class limit of the observed scale can be found by taking $X/\sigma=prf^{-1}(q-.50)$. This is done for the random bred stock and for strain 35 in table 20 and the results plotted against each other in figure 1. With the exception of the boundary between classes 18

and 19 (92.5 per cent white) the values fall rather closely along a straight line. Taking the values at 42.5 and 82.5 as those closest to the standard deviations, considering both scales (fig. 1) we see that a scale interval of 1.533σ in the random bred stock corresponds to one of 1.979σ for strain 35. The corrected standard deviation of strain 35 is thus 77.5 per cent $= \frac{1.533}{1.979}$ of that of the random bred stock and its variance is 60.1 per cent ($= .775^2$)

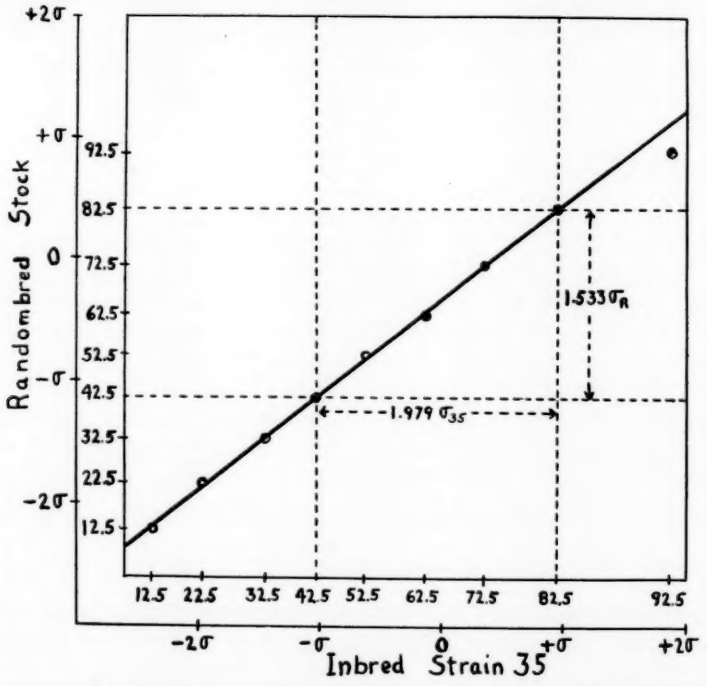

FIGURE 1. Transformation of class limits to give normal distribution of class frequencies in random bred stock plotted against similar transformation for inbred strain 35. The range between 42.5% white and 82.5% white includes 1.979σ in the inbred strain but 1.533σ in the random breds. The variance of the inbred stock is thus 60.1% ($= 1.533^2/1.979^2$) of that of the random bred stock on a scale based on these two points.

of that of the random bred stock. Since the correlation between parent and offspring in the random bred stock was +.199 by the broad category method, the genetic variance (assuming no dominance or epistasis) should be 39.8 per cent and the variance to be expected in an isogenic stock should be 60.2 per cent of that in the random bred stock, or almost exactly the

value actually found in strain 35 by the above method. The agreement with results from the previous method is as close as can be expected. There can be little or no dominance or epistasis among the minor factors which affect spotting.

Putting all of these results together we reach the following approximate analysis of variance in the random bred stock and an isogenic inbred strain.

	Isogenic Inbred Strain	Random Bred Stock
Heredity	0	40
Sex	3	2
Environment		
Age of mother	4	6
Other factors common to littermates	4	
Factors not common to littermates	89	52
	100	100

DISCUSSION

The results fall into a consistent picture. Four classes of factors are indicated.

First is a major pair of alleles, *S,s*. *SS* is usually self but under exceptional conditions shows traces of white up to a small shoulder belt; *Ss* may be self colored or any grade of spotted up to at least 95 per cent white; *ss* is usually well marked with white, often self white, but may have only a trace of white and perhaps may be completely self-colored under very unusual conditions.

Second is a multiplicity of minor genetic factors with additive effects (little or no dominance or epistasis). The median grade of *ss* can be shifted from about 10 to 97 per cent white by the appropriate combination. The median grade of *Ss* may similarly be shifted from self color to about 30 percent white. Finally a little white may appear in *SS* with an extreme array of white modifiers.

Third, there is an enormous amount of non-genetic variability responsible for a range extending from a trace of white to 100 per cent white in isogenic strains whose median grade is near 50 per cent. This can be subdivided into a small portion composed of factors common to littermates, in which is included an effect of age of mother in at least one stock, and a large portion not common to littermates and hence to be interpreted as due to developmental accidents. In a typical random bred stock, all *ss*, about 58 per cent of the variance was due to non-genetic factors, including only 6 per cent common to littermates.

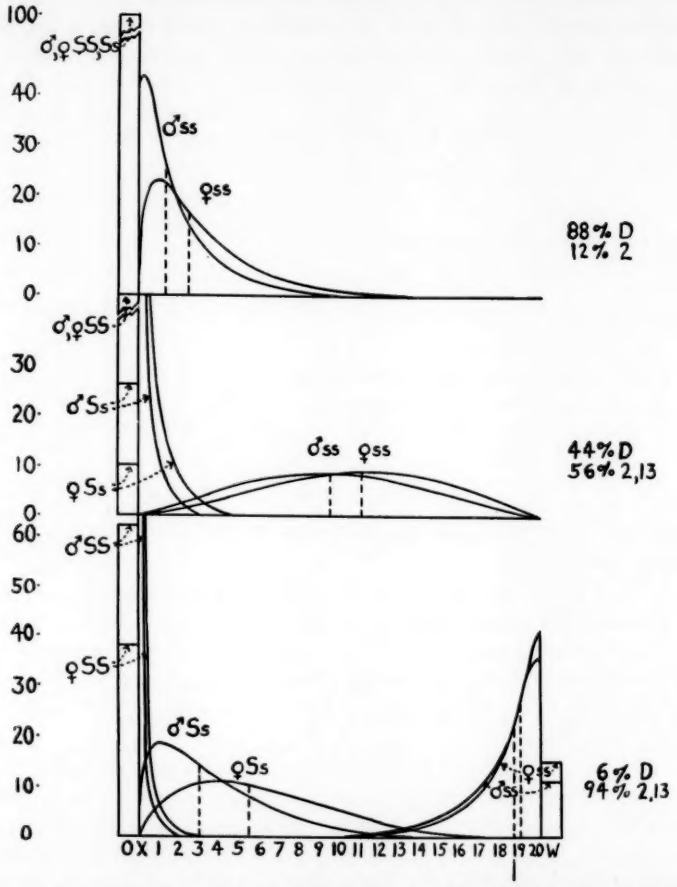

FIGURE 2. Distributions of *SS*, *Ss*, and *ss* according to sex and residual heredity. The abscissas are the classes used in grading. The ordinates are the fitted percentage frequencies in these classes, doubled in the cases of X and 20 to allow for the fact that their ranges are only of half width. Classes O and W are arbitrarily assigned full class ranges. Smooth curves are fitted by the formula $prf^{-1}[\sum f - .50] = 1/.57\,[prf^{-1}(x - .50) - prf^{-1}(M - .50)]$ where prf^{-1} is the inverse probability function, $\sum f$ is the running sum of the fractional frequencies, .57 is the standard deviation (on the transformed scale) of an isogenic stock and M is the median of the distribution to be fitted. The class limits and medians are modified slightly by replacing x by the function $.98x + .0025$ in order to give a class of solid color 0%–.25%) and a class of solid white (98.25%–100%). The medians in the upper series are from 7/8*D* table 7, those in the middle series from (7/8*D*×2), table 7 in the case of *ss* and from (7/8*D*×13), table 8, in the case of *Ss*. The medians in the bottom figure are based on backcrosses (and derivatives) with 7/8 or more of strain 13 (*SS* table 10: *Ss*, *ss* table 9). The distributions so fitted all agree reasonably well with the observed distributions.

Fourth is a sex difference, females having slightly more white than males in all strains, the difference accounting for .2 or 3 per cent of the total variance.

The effects of these four classes of factors are illustrated in figure 2. The variability of males and females of *SS*, *Ss* and *ss*, due to non-genetic factors are shown at the top on a background of modifiers suppressing white, at the bottom with modifiers favoring white, and in the middle with a more typical, intermediate combination of modifiers. The sex difference appears to be greatest in stocks with somewhat less than 50% white in the coat on the average.

We have finally to consider the relation of these results to those of other authors.

Our pair, *S,s*, is doubtless the same as IBSEN'S *S,s* although IBSEN refers to *S* as dominant. IBSEN'S other factors *Fa, fa*; *Na, na*, obviously cannot be used in the interpretation of our results. Judgment as to whether the enormous amount of non-genetic variability of all of our stocks is largely absent from his stocks must await publication of his data.

PICTET'S 4-factor hypothesis also is obviously inapplicable to our data. In our data there is clearly just one major factor with effects on both head and trunk; *ss* typically has much white on both head and trunk, in *Ss* both are reduced while *SS* extracted after many generations of back-crossing to a stock in which both head and trunk are almost wholly white, is usually completely self. The correlation between head and trunk is not perfect, but neither is the correlation between any two areas which could be named, for example, left and right ears, left and right fore feet, shoulder and loin (cf. ILJIN 1928). PICTET has presented no data which justify the conclusion that his stock differs in these respects.

PICTET'S most extensive published data refer to the trunk pattern. How far is our interpretation applicable? It is not possible to give a certain answer. However, it seems possible that the dominant and recessive spotting factors which he found in his two original sets of matings may have been the same factor, *s*, associated with different arrays of modifiers as in our experiments involving 7/8 blood strain 13 and 7/8 blood strain D respectively. With respect to his later generations, derived from crosses of the two foundation stocks, there are so many possible genotypes for every phenotype in both hypotheses and such a wide range of phenotypes for each genotype in ours that it seems not unlikely either could be made to fit. It would require experiments with isogenic stocks to discriminate between them.

Whether there is any non-genetic variability in his stock, that is, a wide range of phenotypes for each genotype, is not considered by PICTET. But the irregularity in localization of spots and frequent asymmetry

which seem to have characterized his stock as well as ours make the existence of such variability probable. If a type of spotting is ever discovered in the guinea pig, which is not subject to non-genetic variability to an important extent, it will probably show something of the orderliness of pattern of hooded rats or Dutch rabbits.

SUMMARY

Analysis of the results of crosses among a considerable number of closely inbred strains of guinea pigs, supplemented by biometric analysis of variability and correlations in random bred and inbred stocks indicate 4 classes of factors as affecting white spotting:

(1) a major pair of alleles *S*,*s* in which *S* (tending toward self) is usually incompletely dominant (statistically) over *s* (tending toward white),

(2) a multiplicity of genes with individually small effects, additive on a suitably transformed scale (no dominance or epistasis),

(3) an enormous amount of non-hereditary variability, not common for the most part even to littermates, but including minor effects of common factors (for example increasing white in young with increasing age of mother),

(4) a sex difference, females having slightly more white than males on the average in all strains.

The interpretations which have been put on white spotting by other recent authors, who have largely ignored the possibility of non-hereditary variability, are shown to be inapplicable to the results described here.

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GENETICAL AND CYTOLOGICAL STUDIES OF A DEFICIENCY (NOTOPLEURAL) IN THE SECOND CHROMOSOME OF DROSOPHILA MELANOGASTER

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THE conclusion that two dominant venation mutants (Plexate and Plexate[2]) and two minutes (Minute-1 and Minute-4) are due to losses from the gene-string—that is, to deficiency—was drawn several years ago on the basis of genetic data and was later confirmed by examination of the salivary chromosomes. Several other genetic deficiencies, notably the Notches, have been similarly located through salivary analysis, by PAINTER, MACKENSEN, MULLER, PROKOFYEVA, DEMEREC and other workers. All of the dominants, especially those which are lethal when homozygous, require checking for chromosomal rearrangements, such as deficiency, duplication, translocation and inversion. An important goal in this work is the establishment of close correspondences between the loci on the genetic maps, deduced from linkage studies, and the particular chromosome localities, deduced from study of the transverse bands which form a diversified series along the salivary chromosomes.

ORIGIN AND CHARACTERISTICS OF NOTOPLEURAL

In the balanced stock of Stubble/*C3,l3a* it was observed by SKOOG (Feb. 20, 1933) that a small percentage of both males and females had shorter, wider, blunter wings, with venation irregularly thickened or branched in a few places. In some individuals there was a break in the posterior crossvein. A Stubble male showing this spontaneous mutant character was outcrossed to Curly (Exp. 610). In F_1 the mutant wing type appeared in about half the progeny of both sexes, hence is an autosomal dominant. Upon inbreeding the F_1 ("Mutant"/Cy; $Sb/3^+$) flies, it was found that all F_2 flies showed both Curly and the "Mutant"; hence "Mutant" has its locus in Chromosome II, and is completely lethal when homozygous. A balanced stock of "Mutant"/Cy, freed of Stubble, was obtained in F_3 and kept. When the "Mutant" was thus separated from Stubble it was found to have itself somewhat shortened bristles, especially the humerals, notopleurals and pretarsals (see fig. 1, hetero-

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zygous female). The shortened bristles proved more reliable for classification than the venation and other wing characters; hence the "Mutant" was renamed Notopleural, with symbol *Np*. There are several other alterations characteristic of the Notopleural type, of which the most obvious is the straggly arrangement of hairs on the thorax. All the Notopleural characteristics are somewhat more extreme in females, and at tempera-

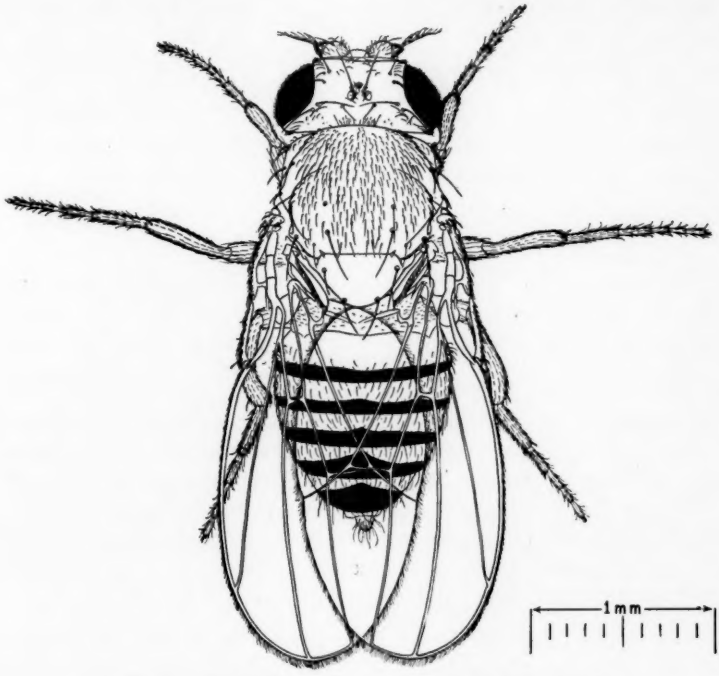

FIGURE 1. Notopleural female heterozygote. (E. M. Wallace, Del.)

tures above 25°C. A majority of *Np* females are completely sterile, while the fertile ones give progenies reduced to half or even to a small fraction of the normal number. The males are fertile and productive. Viability of *Np* is erratic, from practically normal to half-normal. Emergence is delayed somewhat by slow development, but the resulting flies are large and strong in appearance. (In relative valuation the mutant is classed in Rank-4.)

LINKAGE RELATIONS AND LOCUS OF NOTOPLEURAL

Female testcrosses of Notopleural with brown (104.5) and speck (107.0)

gave free recombinations, showing that the locus of *Np* is far to the left of those of *bw* and *sp* (Exp. 610A; 3 fertile, 25 sterile cultures; SKOOG; 117–19; June 7, 1933):

Np	*bw sp*	*Np bw sp*	+	*Np sp*	*bw*	n	*Np-bw R*	*bw-sp R*
105	119	57	66	2	3	352	35.0	1.4%

Next, it was found that the *Np* locus is to the right of Sternopleural (22.0) and of black (48.5) (Exp. 610B; 6 fertile cultures; BRIDGES; 20,370–76; July 19, 1933):

Sp b	*Np*	*Sp Np*	*b*	*Sp Np b*	+	n	*Sp-b R*	*b-Np R*
40	33	9	4	3	3	92	14.1%	6.5%

Further *b* +/+ *Np* linkage data were obtained (Exp. 610C; 3 fertile cultures; BRIDGES; 20,383–85; Aug. 1, 1933):

b	*Np*	*b Np*	+	n	*b-Np R*
106	89	7	12	214	8.9%

Testcrosses of the type *b Np* +/+ + L^2 (Exp. 610D; 14 fertile; BRIDGES; 20,440 ff; Aug. 29, 1933) gave:

b Np	L^2	*b* L^2	*Np*	*b Np* L^2	+	*b*	*Np* L^2	n	*R-1*	*R-2*
468	753	94	76	68	165	19	11	1654	12.1	15.9

From the three foregoing tests in which black and Notopleural were involved, 1986 flies gave 11.4 as the mean percentage of *b-Np* recombinations, with the locus of *Np* between *b* and L^2, at about 60 (48.5+11.5).

More precise localization, as well as more accurate determination of possible crossing over reduction, was made by use of the nearer loci purple (54.5) and engrailed (62.0) in female testcrosses with Notopleural (Exp. 610G; 10 fertile cultures, 3 ♀ ♀ each; SKOOG; 216 ff; Oct. 5, 1933):

pr en	*Np*	*pr Np*	*en*	*pr*	*Np en*	+	n	*R-1*	*R-2*
392	342	13	31	9	2	1	790	5.7%	1.5%

From this testcross the *pr-en* crossing over was 7.2, as compared with the standard 7.5; evidently no marked reduction of crossing over was produced by the Notopleural mutation. The locus of Notopleural was indicated as 1.5 to the left of engrailed (62.0), hence at 60.5, in agreement with the position as deduced from the *b-Np* data.

At this stage cytological examination of the salivary chromosomes (see below) confirmed the hypothesis that Notopleural is due to a deficiency. Since the deficiency is nearly 5 per cent of the length of the right arm of chromosome II, a local reduction in crossing over is expected, hence more precise tests of the linkages were carried out by LI to check this relation.

There are two good characters with loci between purple and engrailed,

namely, cinnabar eye-color (57.8) and bloated wing (59.0). The character bloated (found by P. T. IVES, June 26, 1933), although fairly good (RK2), has been little utilized. For closer study two new stocks were required, namely, *cn en* and *blo en*. To secure *cn en*, *pr en* was crossed to *cn*, and the F_1+ ♀ ♀ backcrossed to *pr en* ♂♂ (Exp. 610J; 7 cultures; LI; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
758	851	103	140	1852	13.1%

Some of the not-*pr en* flies (140) were crossed together in mass cultures. In the next generation they produced *cn en* flies, which were bred together for the required stock.

Similarly, the female testcrosses of *pr*+*en*/+*blo*+ ♀ ×*pr*+*en* ♂♂ gave *pr-en* recombination data and in due course a stock of *blo en* (Exp. 610K; 9 cultures; LI; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
1365	1511	125	148	3149	8.8%

The above two control experiments gave diverse values of 8.8 and 13.1 per cent of recombination for *pr-en*, as compared with standard 7.5. Also, great fluctuation occurred from culture to culture within both tests. Such high variability is rather characteristic of the central regions of chromosomes II and III, and makes comparisons of data of far less value than for other regions.

As a check on the normality of the *cn en* stock, and as a control against the anticipated results with Notopleural, a rather extensive testcross of *cn en*/Oregon-Roseburg (Ore-R) ♀ ♀ was carried out (Exp. 610L; 26 cultures; LI; Feb. 13, 1936):

cn en	+	*cn*	*en*	n	*cn-en R*
4445	4646	322	324	9737	6.6%

A similar control of *blo en*/Ore-R ♀ ×*blo en* ♂♂ (Exp. 610M; 30 cultures; LI; March 1, 1936) gave:

blo en	+	*blo*	*en*	n	*blo-en R*
2772	4793	142	199	7906	4.3%

Finally, a linkage experiment involving *cn en* and Notopleural was carried out (Exp. 610N; 34 fertile cultures; LI; Feb. 14, 1936):

cn en	*Np*	*cn Np*	*en*	*cn*	*Np en*	n	*R-1*	*R-2*
2931	2298	16	23	110	63	5441	0.7%	3.2%

The similar *blo*+*en*/+ *Np*+ testcrosses (Exp. 610P; 36 fertile cultures; LI; March 1, 1936) gave:

blo en	*Np*	*blo Np*	*en*	*blo*	*Np en*	n	*R-1*	*R-2*
450	619_1	0	0	10	19	1098	0.0%	2.6%

These two experiments (*cn en*/*Np* and *blo en*/*Np*) and their controls (*cn en*/Ore-R and *blo en*/Ore-R) show that there is very probably a reduction of crossing over due to the deficiency. There are two measures of the amount of this reduction, namely: $6.6-3.9=2.7$ and $4.3-2.6=1.7$. Supplementary to these, we might give the calculations $4.3-2.9=1.4$ (where 2.9 is the mean value for the three experiments giving *Np-en* data) and $10.3-7.2=3.1$ (where 10.3 is the mean from the two *pr-en* controls and 7.2 is SKOOG's value for *pr-en* as modified by *Np*). The weighted mean from these four measures is about 2.0. But since all the experiments on which this value is based gave approximately 30 per cent more crossing over than expected from the standard map, a value of 1.5 would be more comparable with standard results as the amount of reduction due to Notopleural. Since the genetic length of the right limb of Chromosome II is about 52 units, and the salivary deficiency includes 4.4 percent of the right limb (see below), we might expect that the deficiency would make about 2.3 units reduction, if crossing over is equally distributed. But the general correlation of the linkage maps with the salivary maps shows that crossing over is only about half as free in this particular region as in the right limb as a whole. Hence, only about 1.2 would be the reduction expected, in agreement with the value 1.5 deduced from the experiments. While crossing over is probably entirely eliminated from within the deficient section, it may also be reduced somewhat in the immediate vicinity.

The locus of Notopleural cannot be definitely ascertained by linkage if there is crossing over reduction in the vicinity. If the 1.5 represents the length of the deficient section, the location should be described as extending between two points. These might be approximated at 1.8 to the left of engrailed, or at 60.2, for the right end, and 1.5 further to the left, or at 58.7, for the left end.

CHECK OF NOTOPLEURAL DEFICIENCY FOR POSSIBLE INCLUDED LOCI

In the general neighborhood of Notopleural (58.7–60.2) ±, the standard map (D.I.S. 3) showed the following mutants: staroid (58 ±), bloated (59 ±), engrailed (62.0), upward (62.5 ±) chaetelle (63 ±), Baroid (65 ±), Xasta (68 ±) and Abnormal-wing (65 ± 5). The mutants Baroid, Xasta and Abnormal-wing are associated with translocations. Tests showed that all of these loci are outside the deficiency. The closest neighbor is bloated to the left, and this agrees with the observation that *blo en*/*Np* gave no *blo-Np* recombinations. A more probable position of *blo* is thus at about 58.5.

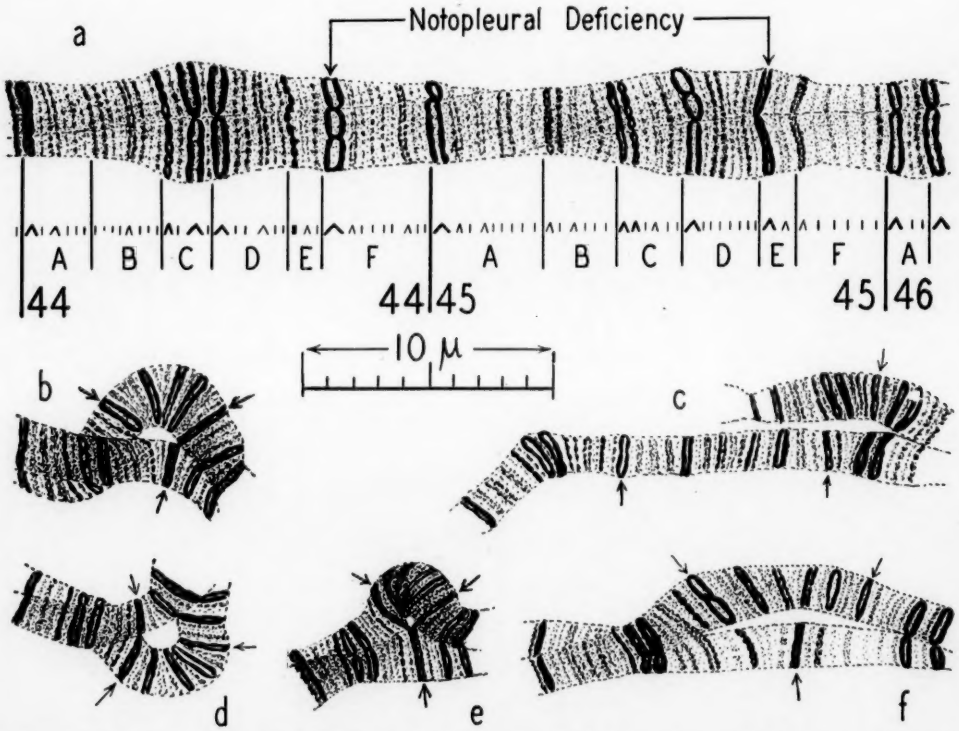

FIGURE 2. Salivary chromosomes of Notopleural heterozygotes. a. Revised normal map of sections 44 and 45 in 2R, with the Notopleural deficiency indicated. b. Synapsis at right limit. c. Non-synapsed strands, both lax. d. Synapsed at left limit. e. Synapsed at both limits. f. Well stretched, non-synapsed deficient strand. (C. B. Bridges, Del.)

SALIVARY ANALYSIS OF NOTOPLEURAL

The first confirmation of the hypothesis that Notopleural is a deficiency was made (Nov. 22, 1935) by LI, who observed a "buckle" and loop at about section 45 in the right limb of Chromosome II (BRIDGES 1935). For a more exact study, numerous permanent preparations of salivary chromosomes of Notopleural/Ore-R females were made. Studies of the normal morphology of sections 44 and 45 were carried out by BRIDGES on Oregon R and numerous other strains. By averaging the measurements and spacings of camera lucida drawings of the five most favorable stretched specimens, a revised map of this region was made (fig. 2a). Easy landmarks to the left of the deficiency are the three heavy doublets in 44 CD, while equally striking markers to the right are the two heavy doublets at the beginning of 46.

Study of the banding (fig. 2c, and especially 2f) showed that the Notopleural chromosome is normal through 44E and from 45E on. But at the rejunction is a doublet not exactly matching any band in the normal chromosome, being less heavy and less separated than the heavy doublet beginning 44F, and heavier than the close doublet beginning 45E. Study of the synapsis relations showed this anomalous band sometimes in synapsis with the 45E1 doublet (fig. 2b) but more frequently in synapsis with 44F1 doublet (fig. 2d) and occasionally with both at once (fig. 2e). Evidently both breaks of the deficiency split through the halves of doublets. The normal map shows 50 distinguishable transverse elements between the breaks of the Notopleural deficiency.

The interpretation to which we incline is that normal 44F1 doublet is a "repeat" (BRIDGES 1935) of two identical bands and that normal 45E1 doublet is similarly a case of identical twin bands. The new band ($\frac{1}{2}$ 44F1 doublet+$\frac{1}{2}$ 45E1 doublet) shows close union of the two parts, suggesting allelism. On this view the two normal doublets would be alleles, one derived from the other, or both modifications of a previous common ancestor.

It is suggested that "repeats" offer a generalized, and perhaps the most frequent, mechanism for further steps in rearrangement—either translocations, inversions or deficiencies—through a preliminary synapsis of homologous or allelic bands or sections, which are carried in separate chromosomes or localities, and subsequent crossing over to give the new configuration. Striking evidence for this general view will be presented in studies of certain other rearrangements.

SUMMARY

The dominant mutant "Notopleural" (symbol *Np*) was found as a spontaneous occurrence by ELEANOR NICHOLS SKOOG, February 20, 1933.

It is characterized by numerous slight departures from the wild type, especially by shortened notopleural, humeral and pretarsal bristles, by straggly microchaetae, by blunter wings with somewhat thickened and branched venation, by low production of eggs by *Np* females and by erratic mortality. The homozygote is completely lethal.

Linkage tests showed the locus to be in the right limb of Chromosome II, between the locus of bloated (58.5) and engrailed (62.0). Crossing over is locally reduced in the presence of *Np* by about 1.5 units. Since *Np* is a deficiency (see below) its limits on the normal map are about 58.7 to 60.2.

Salivary analysis shows a deficiency of 50 recognized bands. Both breaks are between the halves of doublets and the deficient chromosome shows a new doublet composed of the left half of 44E1 heavy doublet and the right half of 45E1 doublet which is slightly less heavy.

It is suggested that these two doublets are homologous, though one or both may have been altered by mutation from their common ancestor. The occurrence of such homologous "repeats" gives a reason for the exchange points or breaks of translocations, inversions and deficiencies coming at particular favored places.

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GENETIC MAPS OF THE AUTOSOMES IN DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

DROSOPHILA PSEUDOOBSCURA is of considerable interest as an object for the study of a number of genetic problems, especially those connected with the hybrid sterility and the methods of race and species formation. As a basis for these studies it is necessary to work out the genetic and the cytological maps of the chromosomes of this species. The present paper deals with the genetic maps of its autosomes.

MATERIALS AND METHODS

Since the two races of *Drosophila pseudoobscura* are different in gene arrangement—they differ in an inversion in each limb of the X chromosome and an inversion in the second chromosome (TAN 1935a,b, KOLLER 1936)—and since strains of the same race from different geographic origins may also differ in gene arrangement, it becomes imperative for the purpose of this work to use always a definite standard strain. Race A strain from Georgetown, Texas, was chosen.

All mutant stocks of race A *D. pseudoobscura* kept in this laboratory were used. The author is indebted to Miss BEERS of the University of Southern California for the use of some mutants she discovered. Some mutant stocks were sent here from the Genetics laboratory of Edinburgh University, Scotland, through the kindness of Drs. KOLLER and CREW, to whom the author wishes to express his appreciation. In the course of the investigation a few new mutants were discovered by the author and some of them proved valuable because of their favorable locations. Prof. STURTEVANT kindly let me use some of the new mutants he had found.

For linkage experiments all cultures were raised in an incubator at $25 \pm .5°C$.

DESCRIPTION AND LOCALIZATION OF MUTANT GENES

Since the present paper is confined to the autosomes of *D. pseudoobscura*, only the autosomal mutants will be briefly described below. In each chromosome mutants will be described more or less in the order of their discovery.

The second chromosome

Bare (*Ba*). Bare was discovered by STURTEVANT (1934) in race A. It is characteized by the shortening of all the macrochaetae. The character can be easily distinguished from the wild type, and the viability and productivity of the heterozygous flies are excellent. Homozygous Bare flies are occasionally viable.

Smoky (*Sm*). Smoky, another dominant gene in this chromosome, was found by Miss BEERS (1934) in race B. It has been successfully transferred to race A. The mutant gene causes the thickening and branching of wing veins, especially the second longitudinal vein. The minimum expression is

TABLE 1

Two point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		CROSSOVERS		TOTAL
		Chromosome II				
bi Ba	*bi*×*Ba*	135	145	21	7	308
		Chromosome III				
Sc cv	*Sc*×*cv*	181	175	141	158	655
		Chromosome IV				
in j	*in j*×+	199	177	59	26	461
in j	+×*in j*	354	341	54	73	822
in Cy	*in*×*Cy*	128	104	117	119	468
j tg^3	+×*j* tg^3	149	157	103	112	521
j Cy	*Cy*×*j*		886		235	1121
tg^3 *Cy*	tg^3 *Cy*×+	261	288	30	54	633

the formation of a small delta-like structure at the distal end of the second longitudinal vein. It is invariably lethal when homozygous. Smoky also has the effect of roughening the surface of the eyes. By crossing to Bare and backcrossing the F_1 Bare Smoky females to wild type males, it was found that the crossing over frequency between the two exceeds 40 per cent.

Glass (*gl*). The recessive mutant glass was discovered and described by CREW and LAMY (1935a). The eye is reduced in size and surrounded by a smooth colorless rim. The pigment in the central space appears to be greatly reduced in amount and leaves only a pinkish or reddish hue. The character can be easily recognized with the naked eye. The viability and fertility are about as good as in wild type. In a cross between *Sm Ba*/*gl* ♀ ×*gl* ♂, the result (table 2) shows crossing over values of 42.0 per cent between Bare and Smoky, 18.9 per cent between Bare and glass, and 46.3 per cent between Smoky and glass. Since the smallest number of flies were represented by Bare-glass and Smoky classes, which must be double cross-

overs, the sequence of the three genes in the chromosome must be Smoky-Bare-glass, Bare being nearer to glass than to Smoky.

Bithorax (*bi*). The gene bithorax was first reported by CREW and LAMY (1934b) as an autosomal recessive mutant, causing an enlargement of the balancers. In extreme cases, the balancers may take the form of wing-like organs. The mutant flies are relatively weak and their viability is low. Sometimes the character may overlap normal.

DONALD (1936) described the mutant in detail and reported that the locus of bithorax is 25 units from glass. My data from the cross *bi gl/Sm Ba* ♀ to *bi gl* ♂ (table 3) show that bithorax is 28.9 units from glass. Between

TABLE 2

Three point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE CROSSOVERS				DOUBLE C.O.		TOTAL
				REGION 1		REGION 2		1, 2		
				Chromosome II						
Sm p ps	*Sm p ps*×+	134	59	50	95	81	45	39	39	542
Sm Ba gl	*gl*×*Sm Ba*	90	164	137	52	36	27	20	20	546
bi p Ba	*bi p*×*Ba*	226	206	20	9	7	5	4	4	481
bi p ps	*bi p ps*×+	223	138	10	9	165	89	7	4	645
bi gl ps	+×*bi gl ps*	119	21	44	21	24	10	6	5	250
				Chromosome III						
or Bl Sc	*Bl Sc*×*or*	160	175	19	13	34	39	1	1	442
or ab pr	*or ab pr*×+	251	146	9	29	182	94	7	18	736
or Sc pr	*or Sc pr*×+	74	45	17	19	23	17	5	6	206
				Chromosome IV						
in j tg^3	*in j tg*^{3}×+	172	90	21	59	70	104	13	5	534
tg^3 *Cy Ro*	*Ro*×*tg*^{3} *Cy*	157	160	19	11	5	2	0	0	354

glass and bithorax lies the gene Bare, which gives 21.2 per cent of recombinations with glass on one side and 9.7 per cent with bithorax on the other (table 4).

Pink (*p*). The gene for pink eye color was found in a cross of the La Grande-2 strain to orange Scute purple by STURTEVANT in 1934. It is recessive and located near Bare. Pink itself is variable and sometimes overlaps wild type. However, in combination with orange, a third chromosome gene, it can be easily classified. As a matter of fact, orange alone gives a bright red eye color (like vermilion), while in combination with pink it gives a true orange color.

According to STURTEVANT, pink is located close to Bare and the two gave only a few per cent of crossing over. An experiment involving the cross of *bi p/Ba* ♀ with *bi p* ♂ gave 7.7 percent of crossing over between

bithorax and pink and 4.2 per cent between pink and Bare (table 2). The sum of the two, 11.9, is somewhat higher than the value, 9.7 (table 4), that was obtained directly between bithorax and Bare. Despite the unexpectedly high number of observed double crossovers, it is justifiable to put the gene pink between bithorax and Baie.

Pauciseta[1] (*ps*[1]) *and its allele pauciseta*[2] (*ps*[2]). The gene pauciseta[1] (*ps*[1]) was discovered by Miss GROSCURTH in the Chehalis-4 strain (race A), several generations after this strain had been derived from a single female caught in nature. The mutant is characterized by the absence of some bristles, especially the anterior dorso-centrals and the scutellars. In some cases, however, all bristles may be present, but one or both anterior dorso-centrals become somewhat more slender than normal. Unless the bristles are carefully examined, pauciseta[1] can be easily overlooked.

Pauciseta[2] was found by the author as a single female in progeny of a cross between *ct y m sn v se/s*[2] ♀ with *ct y m sn v se*♂ in March 1936. The fly had some bristles missing on both the thorax and the scutellum. It was mated with wild type males and produced exclusively wild type flies in F_1, which in F_2 produced 365 wild type and 122 pauciseta[2]. When pauciseta[2] males were mated to bithorax glass pauciseta[1] females, the progeny showed the pauciseta character, indicating that the new pauciseta is an allele of the old one. Since the two are indistinguishable, the new pauciseta may be designated as *ps*[2].

To localize the gene pauciseta in the second chromosome, two sets of experiments were carried out. They were *bi p ps/+* ♀ by *bi ps ps*♂ and *bi gl ps/+* ♀ by *bi gl ps*♂. The result as summarized in table 2 enables us to localize the gene pauciseta on the right end of the second chromosome.

The third chromosome

Orange (*or*). Orange eye color was found by LANCEFIELD and later described by CREW and LAMY (1934a). It is recessive and cannot be distinguished from the sex-linked vermilion eye color. The mutant can be easily classified even in combination with most other eye colors. The viability and productivity of the mutant fly are as good as in normal.

Purple (*pr*). The gene purple, another recessive eye color mutant, was found by CREW and LAMY (1932). It is a translucent color ranging from yellowish brown to chestnut. In males the testicular sheath appears colorless. When it is combined with orange, the eye becomes grayish white in color.

Scute (*Sc*). Scute, a dominant gene causing the absence of most bristles on the thorax and the head, was also reported by CREW and LAMY (1934a). The homozygous forms of Scute can be distinguished from the heterozygotes by the rough eyes and the absence of some microchaetae. Accord-

TABLE 3

Four point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE C.O.						DOUBLE C.O.						TRIPLE C.O.		TOTAL
				REG 1		REG 2		REG 3		1, 2		1, 3		2, 3		1, 2, 3		
										Chromosome II								
Sm bi Ba gl	*bi gl*×*Sm Ba*	186	210	199	100	17	20	78	45	28	13	60	28	1	7	7	1	1000
										Chromosome III								
or ab Ja pr	*or ab pr*×*Ja*	286	146	14	43	44	24	108	45	4	8	4	4	6	2	1	2	741
or Ja Sc pr	*or Sc pr*×*Ja*	205	152	36	37	15	10	42	54	1	1	5	5	4	3	0	5	575
or Ja pr cv	*or pr cv*×*Ja*	273	202	39	40	85	98	59	68	12	7	17	4	7	8	2	1	922

ing to CREW and LAMY (1934a) Scute is located half way between orange and purple, each of which gives about 25 per cent of crossing over with Scute. Due to its favorable location and its clear cut expression, Scute has proved to be an extremely valuable mutant in this group.

Crossveinless (*cv*). The mutant crossveinless was reported by CREW and LAMY (1934b) as an autosomal recessive, which causes the absence of the posterior crossvein. The anterior crossvein may either be absent or incomplete. The mutant is easily distinguishable, if the wing is not damaged. According to DONALD (1936) crossveinless is located 17 to 26 units from

TABLE 4

Total data for each pair of loci.

CHROMOSOME II			CHROMOSOME III			CHROMOSOME IV		
LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED	LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED	LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED
Sm bi	43.6	1000	*or Bl*	7.7	442	*in j*	17.1	1817
Sm p	41.2	542	*or ab*	9.7	1477	*in lg*	47.6	534
Sm Ba	22.8	1546	*or Ja*	15.8	2235	*in Cy*	50.1	468
Sm gl	46.5	1546	*or Sc*	21.8	1223	*j lg*	38.6	1055
Sm ps	50.0	542	*or pr*	36.7	3180	*j Cy*	41.9	1121
bi p	5.9	1126	*or cv*	42.5	922	*lg Cy*	11.5	987
bi Ba	9.7	1789	*Bl Sc*	17.0	442	*lg Ro*	10.5	354
bi gl	29.2	1250	*ab Ja*	12.3	741	*Cy Ro*	2.0	354
bi ps	41.6	895	*ab pr*	36.7	1477			
p Ba	4.2	481	*Ja Sc*	6.8	575			
p ps	39.6	1187	*Ja pr*	23.6	1497			
Ba gl	21.2	1546	*Ja cv*	38.0	922			
gl ps	18.0	250	*Sc pr*	21.6	781			
			Sc cv	45.6	655			
			pr cv	18.0	922			

purple. This is in close agreement with my data, which give 18.0 per cent of crossing over between purple and crossveinless (table 4).

Jagged (*Ja*). Jagged wing is a dominant mutation found by the author. Usually, only the inner margin of the wing is notched. In extreme cases, the whole wing may become strap-like and bear notches on all margins. Three such flies, two females and one male, appeared in a cross between *bi gl/Sm Ba* ♀ and *bi gl* ♂ in December 1935. A single Smoky Bare Jagged female was successfully crossed to three wild type males, producing 52 Jagged and 63 non-Jagged flies, equally distributed among both sexes. This shows that Jagged is an autosomal dominant mutation.

The test for the viability of the flies homozygous for Jagged wing indicates that Jagged is lethal when homozygous.

The locus for Jagged has been determined, by the combined results of

several different type of four-point crossing over experiments (table 3), to lie 6.8 units to the left of the gene Scute (table 4).

Abrupt (*ab*). Abrupt longitudinal vein is a recessive mutant found by the author. It produces a shortening of the fourth longitudinal vein. In extreme cases, the vein may abruptly stop just below the posterior crossvein. The mutant cannot be distinguished from either short, a sex-linked gene causing the shortening of the third and fourth longitudinal veins, or short-4 which was described by CREW and LAMY (1935a) to produce the shortening of the fourth and fifth longitudinal veins. In order to avoid confusion, separate names are here proposed to designate these mutants. Short (*s*) remains to designate the sex-linked one. The fourth chromosome one, originally known as short-4 (s_4), is renamed incomplete (*in*). The name abrupt (*ab*) applies to the one in the third chromosome, which is now under discussion.

The origin of abrupt can be traced back to the orange purple stock. In three cultures of *na/or Sc pr* ♀ by *or pr* ♂, there appeared several males whose wings were indented at their inner margin. The gene concerned was found to be allelomorphic to the beaded originally described by LANCEFIELD (1922). It is known as bd^2. From a cross between *or* bd^2 and *or* sibs, three *or pr* females appeared to have their fourth longitudinal vein shortened. By mating them to wild type sibs, a good many orange abrupt purple flies of both sexes were obtained. Since all abrupt flies had orange purple eye color, the mutant must have originated in the *or pr* stock. When *or ab pr* flies were mated to incomplete (*in*), all F_1 individuals were wild type, indicating that abrupt and incomplete are not allelomorphs. An *or ab pr* stock was soon established. At the beginning some abrupt flies appeared to overlap normal. But after several generations of selection and inbreeding, the character became more pronounced and at the same time bred true.

The locus for the gene abrupt has been found to lie between orange and Jagged. As shown in table 4, it is closer to the former (9.7 units) than to the latter (12.3 units).

Blade (*Bl*). Blade wing is a dominant discovered by STURTEVANT (unpublished). The wing assumes a blade-like shape. It is easily classifiable and has normal viability and productivity. STURTEVANT crossed *or/Bl Sc* ♀ to *or* ♀ and found Blade to give 7.7 per cent of crossing over with orange and 17.0 per cent with Scute. With his permission the result of this cross is also included in table 2.

The fourth chromosome

Curly (*Cy*). Curly, a dominant gene, was discovered by Miss BEERS (1934) in race B. The wing may be curled either upward or downward.

Flies homozygous for Curly are always inviable. In heterozygous condition, the mutant fly is fully viable and fertile. Curly can be easily distinguished from normal. The gene has been successfully transferred from race B to race A.

Jaunty (*j*). The gene jaunty was reported by CREW and LAMY (1935a). It is recessive, and the mutant flies show a slight upturning of the tip of the wing. Occasionally, it overlaps normal.

Incomplete (*in*). The gene incomplete, another fourth chromosome recessive found by CREW and LAMY (1935a), was originally described by them as short-4. According to them incomplete and jaunty gave about 10 per cent of crossing over. Recently, DONALD (1936) reported that the two gave 15 to 16 per cent of recombinations. My data as shown in table 4, giving 17.1 per cent of crossing over, is in close agreement with the result of DONALD.

Multiple alleles of tangled (*tg*). The first mutant of tangled, tangled[1] (*tg*[1]), was discovered by CREW and LAMY (1934b), who described it as fused. Later (1935a) they changed the name to tangled. It is recessive; in the mutant flies the second and third longitudinal veins come together at their distal ends, often with extra crossveins. The same also occurs with the fourth and fifth longitudinal veins. In extreme cases, the wing may be tilted up.

A single tangled[2] male, similar to tangled[1], except for having few extra veins, was found in Bare orange Curly stock. It was mated to wild type females, and in F_2 several *Ba or Cy* *tg*[2] and *or* *tg*[2] flies were obtained.

A single tangled[3] male fly found in the Curly stock had only one extra crossvein connecting the distal ends of the second and the third longitudinal veins. It was mated to wild type females, giving in F_1 all wild type flies and in F_2 8 *tg*[3] and 435 wild type ones. The great excess of wild type flies over the mutant type may be accounted for by presence of some modifier in the wild type parent, for after several generations of selection, *tg*[3] started to breed true.

A fourth allele of tangled, *tg*[4], was obtained by Dr. STURTEVANT in the echinus stock. The mutant flies resemble very much either *tg*[1] or *tg*[2]. When they were crossed to each of the other three tangled alleles, all offspring were tangled.

Among the four alleles of tangled, *tg*[3] was most favorable for experimental purposes, because *tg*[3], having only one clear extra crossvein instead of the many characteristic of *tg*[1], *tg*[2], or *tg*[4], can be distinguished in combination with other fourth chromosome wing mutants. Moreover, tangled[3] wings, unlike other alleles of the same locus, are never tilted at the tip. Hence, for genetic analysis of the locus, *tg*[3] flies have been almost exclusively used.

According to DONALD (1936) tangled and jaunty gave 44 to 49 per cent of crossing over and the sequence of the genes was assumed to be incomplete-jaunty-tangled. My data, given in table 4, are in agreement with DONALD's in regard to the sequence of genes but show less crossing over between *in* and *j*. This difference is probably due to the fact that the tangled used by DONALD was tg^1, which, being more extreme, may obscure the classification of flies in the combination of tangled and jaunty.

Rough (*Ro*). Rough is a dominant eye mutation found by Miss GROSCURTH in race A. It causes irregular arrangement of eye facets. Not infrequently the mutation overlaps normal. A single experiment involving the cross of tg^3 *Cy*/*Ro* ♀ by tg^3 ♂ gave 9.5 per cent of crossing over between tg^3 and *Cy* and 2.0 per cent between *Cy* and *Ro*. The absence of *Ro Cy* and tg^3 flies indicates that *Ro* is located to the right of *Cy*.

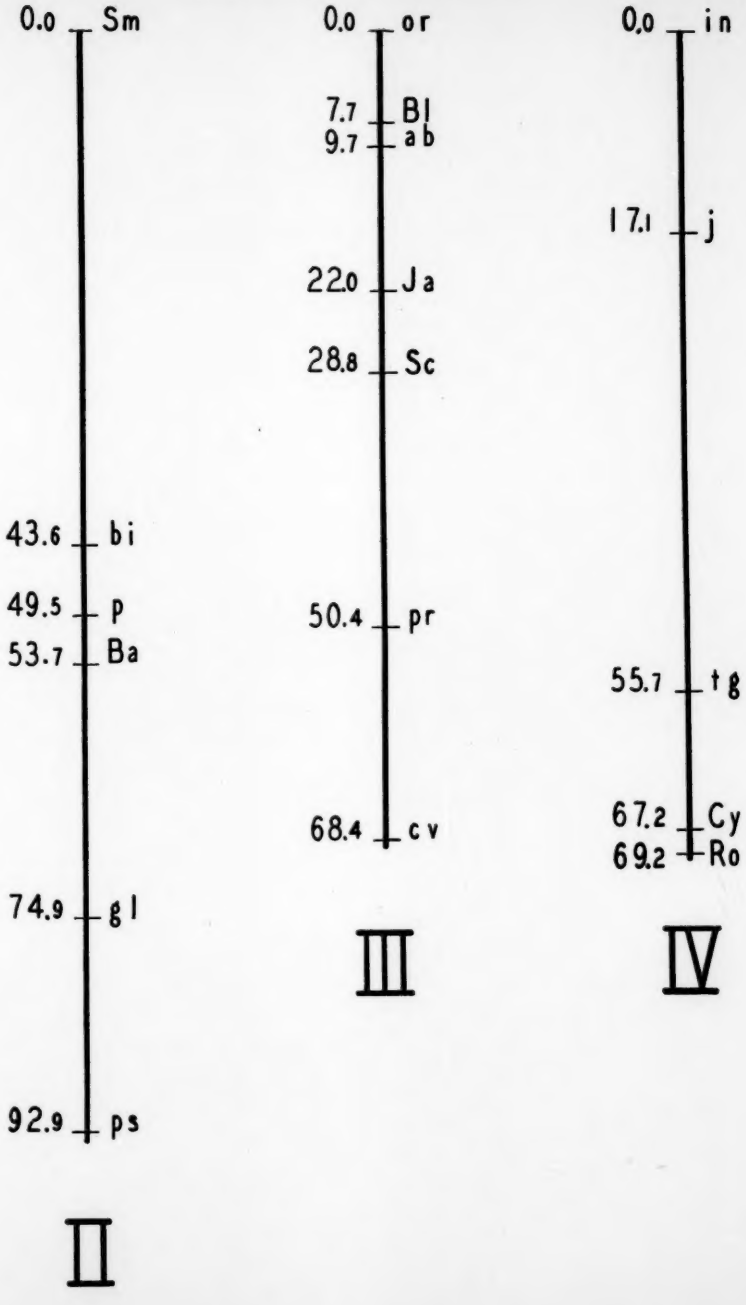
CONSTRUCTION OF THE GENETIC MAPS

According to MORGAN, STURTEVANT and BRIDGES (1925) four autosomal groups of genes were reported by LANCEFIELD with 4, 6, 1 and 2 recognized loci respectively. These, together with the sex-linked group, made the total number of linkage groups 5, which is equal to the haploid chromosome number of the species. In spite of the facts that the autosomal linkage groups reported by LANCEFIELD were imperfect, and that most of his mutants are now lost, some of them have been found to be the same as some of the ones described above. For this reason, the numbering of the second and the third linkage groups still follow the system of LANCEFIELD. The numbering of the fourth linkage group is justified on the basis of the cytological evidence which shows that the representative gene of this group is located in the rod-shaped chromosome and not in the chromosome now designated as the fifth, in which no gene is represented here.

A summary of linkage data is presented in tables 1–3. The first column at the left shows not only the loci concerned but also their sequence. Under the column headed "type of cross," types of two parents involved in the cross are shown, the one on the left being female and the one on the right male. In the third and the following columns classes are entered under the headings indicating the type of crossing over they represent. In every case the class which includes the individuals bearing the wild type allele of the most left-hand locus concerned is placed first, and is followed by the contrary class. The results are, of course, obtained from the backcrosses of the F_1 hybrids to multiple recessives.

Table 4 shows the total data for each pair of loci in the second, third and four chromosomes respectively. The resulting maps are shown in figure 1.

It is, of course, realized that these maps are only preliminary ones, and


0.0 Sm
43.6 bi
49.5 p
53.7 Ba
74.9 gl
92.9 ps
II
0.0 or
7.7 Bl
9.7 ab
22.0 Ja
28.8 Sc
50.4 pr
68.4 cv
III
0.0 in
17.1 j
55.7 tg
67.2 Cy
69.2 Ro
IV

that both the total map-lengths and the distance between the genes may prove to be larger than here indicated. The data presented in table 4 are in general self consistent. Several apparent discrepancies may, however, be noticed; for instance, the value shown between Smoky and bithorax is slightly higher than between Smoky and pink or Smoky and Bare, although the maps show the reverse relation. Similar discrepancies are observed in the values between crossveinless and Scute and between crossveinless and Jagged. These discrepancies are probably due to differential viability of the classes concerned in different experiments.

In determining the map distances, the genes lying nearest to the spindle fibre attachment in each chromosome are taken as the zero points or the left ends. The evidence leading to the determination of the locations of the genes in relation to the spindle fibres will be published elsewhere in connection with the cytological maps. Suffice it to mention here that the loci known to lie nearest to the spindle fibre attachments are Smoky (*Sm*) in the second, orange (*or*) in the third, and incomplete (*in*) in the fourth chromosome. Hence, these loci are taken as zero points.

Recently, CREW and LAMY (1935a) attempted to homologize the second chromosome of *D. pseudoobscura* with the left arm of the V-shaped third chromosome of *D. melanogaster*, and the fourth chromosome of *pseudoobscura* to the right arm of the V-shaped second chromosome of *melanogaster*. In view of the facts that relatively few genes are now known in *D. pseudoobscura* and that the two species cannot be crossed to effect a direct test, it seems to the author that attempts to establish the homology of genetic maps of the two species are rather hazardous. Indeed, DOBZHANKSY and TAN (1936) show that even the two closely related species, *D. pseudoobscura* and *D. miranda*, have no single chromosome in common. *D. pseudoobscura* and *D. melanogaster*, being much less closely related, have the gene arrangement in their chromosomes altered beyond recognition.

The author wishes to express his gratitude to Professors TH. DOBZHANSKY and A. H. STURTEVANT for encouragement, advice and use of their stocks.

SUMMARY

1. The mutants belonging to the three autosomal linkage groups of *Drosophila pseudoobscura* are briefly described.

2. The genetic maps of the three autosomes of the species are constructed as shown in figure 1.

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PHENOGENETIC STUDIES IN SCUTE OF D. MELANOGASTER
III. THE EFFECT OF TEMPERATURE ON SCUTE 5

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INTRODUCTION

THIS paper deals with the quantitative variations, at different temperatures, in the bristle numbers of *sc 1*, *sc 5*, and their compound *sc 1/sc 5*. The allele *sc 5* was found by GAISSINOVITSCH (1930). According to DUBININ (1929, 1932), *sc 5* is a less extreme allele than *sc 1*. (1) *Sc 5* effects fewer bristles and has a lesser effect on those bristles. (2) The bristles affected by both *sc 1* and *sc 5* are the only ones affected in the compound *sc 1/sc 5*. (3) The mean bristle numbers of the compound are intermediate between *sc 1* and *sc 5*.

It will be shown in this paper that these generalizations do not hold at all temperatures for all the bristles. These experiments further add to the growing amount of quantitative data which ultimately must be used to formulate definitely and test a quantitative theory of development such as proposed by GOLDSCHMIDT (1927) and PLUNKETT (1926).

MATERIALS AND METHODS

Culture conditions

The usual Drosophila culture methods were employed (banana-agar gel with added yeast) with added precautions to insure uniform conditions for large numbers of flies raised in half-pint bottles (CHILD 1935). To insure that the *sc 5*, *sc 1*, and the *sc 1/sc 5* flies had as similar an environment as possible, they were raised in the same culture in most of the experiments. Two methods were used to obtain the various genotypes in the same bottles. In some experiments males and females of both alleles were mated in the same bottle. In other experiments *sc 1/sc 5* flies were mated to *sc 1* and *sc 5* males in the same bottle.

Flies 4–7 days old raised under favorable conditions were used for egg laying. About 20–30 females were used per bottle with an egg-laying period of from 1 to 3 hours duration. The temperature was regulated in incubators held constant to within 0.1°.

Selection

The details of selection of the *sc 1* stock and the tests for the adequacy of selection were presented in a previous communication (CHILD 1935).

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The *sc 5* flies were made isogenic with the *sc 1* by twenty generations of backcrossing *sc 1/sc 5* by *sc 1* males. The only difference between these *sc* stocks is, therefore, the possession of one or the other of the *sc* alleles.

The original *sc 5* stock was actually *y sc 5*. During the course of these experiments one crossover was obtained between *y* and *sc 5*, thus eliminating the yellow. The bristle numbers of *y sc 5* and *sc 5* were found to be the same, however, and in reporting these data no distinction will be made as to whether the flies were yellow or wild type for color.

EXPERIMENTAL DATA

The flies were raised for their total period of development at temperatures ranging from 14° to 30°. The complete and more extensive data relat-

TABLE 1*

Effect of Temperature on mean bristle numbers.

Males

$T \pm 0.1°$	*M a sc*		*M p sc*		*M i v*	
	sc 5	*sc 1*	*sc 5*	*sc 1*	*sc 5*	*sc 1*
14°	.11±.019	.020±.014	.24±.027	.060±.024	.62±.030	.68±.046
15°	.11±.023	.019±.004	.26±.032	.035±.005	.72±.033	.94±.029
16°	.10±.015	.027±.012	.14±.018	.049±.016	.91±.015	.99±.005
18°	.09±.018	.004±.002	.57±.014	.005±.002	.99±.006	1.
19°	.13±.036	.012±.012	.69±.015	.002±.001	1.0	1.
20°	.18±.036	.005±.001	.86±.026	.0	1.	1.
22°	.19±.033	.005±.003	.69±.021	.0	1.	1.
23°	.21±.017	.025±.014	.09±.004	.0	1.	1.
24°	.35±.036	.020±.014	.11±.005	.0	1.	1.
25°	.22±.022	.003±.002	.00	.0	1.	1.
26°	.28±.025	.008±.008	.06±.004	.0	1.	1.
27°	.27±.024	.007±.003	.00	.0	1.	1.
28°	.28±.025	.004±.002	.06±.004	.0	1.	1.
29°	.32±.023	.005±.002	.05±.004	.0	1.	1.
30°	.17±.016	.000	.22±.007	.0	1.	1.

* a sc, p sc, anterior and posterior scutellars; iv, pv, inner and posterior verticals; oc, ocellar.

ing to the effect of temperature on the mean bristle numbers of *sc 1* have been reported previously (CHILD 1935). The *sc 1* flies in these experiments served, therefore, as controls. Those few experiments were discarded in which the bristle numbers of the *sc 1* flies differed from the results previously obtained. This type of control served as a very careful check on the temperature and the culture conditions.

The results of these experiments are presented in tables 1 and 2, and are illustrated in figures 1 and 2. At each temperature, between 50 and 300 flies of each type were counted. The results are given as mean bristle

TABLE 2

Effect of temperature on mean bristle numbers.

Females

$T \pm .1°$	*M p sc*			*M pv*		
	sc 5/sc 5	*sc 1/sc 5*	*sc 1/sc 1*	*sc 5/sc 5*	*sc 1/sc 5*	*sc 1/sc 1*
14°	.54 ± .054	.47 ± .033	.28 ± .033	1.	.93 ± .017	.020 ± .019
15°	.54 ± .048	.42 ± .049	.23 ± .012	1.	.98 ± .014	.060 ± .006
16°	.46 ± .035	.35 ± .028	.15 ± .025	1.	.97 ± .010	.045 ± .015
18°	.36 ± .029	.20 ± .028	.07 ± .077	1.	.95 ± .015	.077 ± .088
19°	.34 ± .034	.18 ± .041	.04 ± .025	1.	.95 ± .023	.036 ± .025
20°	.25 ± .036	—	.02 ± .001	1.	—	.086 ± .008
22°	.09 ± .024	.09 ± .031	.01 ± .004	1.	.97 ± .018	.045 ± .009
23°	.07 ± .015	.08 ± .019	.01 ± .002	1.	.98 ± .009	.028 ± .014
24°	.09 ± .023	.04 ± .016	.00	1.	.98 ± .009	.000
25°	.06 ± .018	.04 ± .020	.01 ± .009	1.	.96 ± .020	.012 ± .003
26°	.04 ± .015	.02 ± .011	.00	1.	.94 ± .018	.009 ± .009
27°	.05 ± .017	.00	.00	1.	.97 ± .013	.000
28°	.06 ± .016	.01 ± .009	.00	1.	.96 ± .018	.001 ± .001
29°	.05 ± .012	.03 ± .012	.00	1.	.78 ± .031	.000
30°	.06 ± .015	.03 ± .011	.00	1.	.74 ± .026	.000

$T \pm .1°$	*M oc*			*M a sc*		
	sc 5/sc 5	*sc 1/sc 5*	*sc 1/sc 1*	*sc 5/sc 5*	*sc 1/sc 5*	*sc 1/sc 1*
14°	1.	.93 ± .017	.00	.14 ± .037	.18 ± .025	.04 ± .026
15°	1.	.94 ± .024	.00	.13 ± .035	.18 ± .038	.09 ± .008
16°	1.	.96 ± .011	.00	.20 ± .028	.12 ± .019	.08 ± .021
18°	1.	.98 ± .009	.00	.25 ± .027	.23 ± .028	.09 ± .009
19°	1.	.99 ± .011	.00	.38 ± .035	.39 ± .052	.07 ± .035
20°	1.	—	.00	.49 ± .042	—	.13 ± .003
22°	1.	.99 ± .011	.00	.61 ± .039	.50 ± .054	.17 ± .006
23°	1.	1.	.01 ± .007	.63 ± .028	.55 ± .034	.22 ± .031
24°	1.	1.	.04 ± .018	.68 ± .037	.58 ± .033	.24 ± .040
25°	1.	1.	.08 ± .008	.69 ± .033	.51 ± .050	.17 ± .011
26°	1.	1.	.40 ± .050	.64 ± .036	.50 ± .038	.12 ± .033
27°	1.	1.	.41 ± .021	.61 ± .038	.46 ± .039	.14 ± .015
28°	1.	1.	.60 ± .013	.62 ± .033	.50 ± .044	.06 ± .006
29°	1.	1.	.75 ± .017	.61 ± .029	.50 ± .037	.05 ± .009
30°	1.	1.	.85 ± .020	.52 ± .030	.45 ± .030	.05 ± .009

$T \pm .1°$	*M i v*		
	sc 5/sc 5	*sc 1/sc 5*	*sc 1/sc 1*
14°	.94 ± .022	.93 ± .018	.98 ± .019
15°	.99 ± .01	1.	1.
16°	1.	1.	1.

M. i.v. = 1 at higher temperatures.

numbers per half fly. The *sc 1* curves contain additional points from data previously obtained (CHILD 1935).

DISCUSSION OF RESULTS

With one exception, the i.v.,[1] the bristle numbers of the *sc 5* stock are higher than the *sc 1*. With respect to the i.v. bristle, *sc 5* is therefore a more extreme allele than *sc 1* (fig. 2). Furthermore, as pointed out previously (CHILD 1935) this variation in the M. i.v. is contrary to the subgene hypothesis (DUBININ 1929, 1932, GAISSINOVITSCH 1930), since the i.v. is in a center outside both the *sc 1* and *sc 5* centers as postulated by the Russian workers.

It is further evident from these data that the mean bristle number of a particular bristle (if at all variable) varies in the same way with temperature in the *sc 1*, *sc 5*, and *sc 1/sc 5* stocks.

The bristle numbers of the *sc 1/sc 5* are intermediate between *sc 1* and *sc 5*, although this relationship is somewhat obscured at low temperatures for the asc. The mean bristle numbers of *sc 1/sc 5*, moreover, are closer to those of *sc 5* than *sc 1*. In other words, *sc 5* appears to be "dominant" over *sc 1*. However, the degree of this dominance is not the same for all bristles, and, furthermore, it is not the same for the same bristle at different temperatures.

The data on the pv and the oc bristles (these should not have varied in *sc 1/sc 5* according to subgene hypothesis) illustrate a problem which has been recognized by Drosophila workers on other characters; PLUNKETT (1926) on Dichaete, MOHR (1932) on vestigial, BARON (1935) on eyeless, TIMOFEEF-RESSOVSKY (1934) on venation, and others. This point can be best made clear by citing specific examples. MOHR (1932) found that vg^{ni}/vg^{ni} (nicked) and the wild type are alike phenotypically, both giving normal wings. This, however, does not imply that the concentration of some substance or substances producing the character, normal wings, is the same in both genotypes. This was shown by comparing nicked and the normal allele in combination with the other mutant alleles of vestigial. In combination with *vg* alleles, the character nicked could be discerned. Other slight *vg* alleles showed a similar behavior, their expression depending also upon environmental conditions, especially temperature. BARON (1935) in analyzing isogenic populations of *ey* (eyeless) drosophilae found that there was a variation in the amount of eye producing substance among the "no-eyed" eyeless, although all the "no-eyed" flies had less than the amount needed to produce eyes.

At Minnesota with Dr. HUTT (data unpublished) I found a similar situation in a character in Gallus known as "down defect," a bald spot

[1] For meanings of the bristle abbreviations see footnote to table 1.

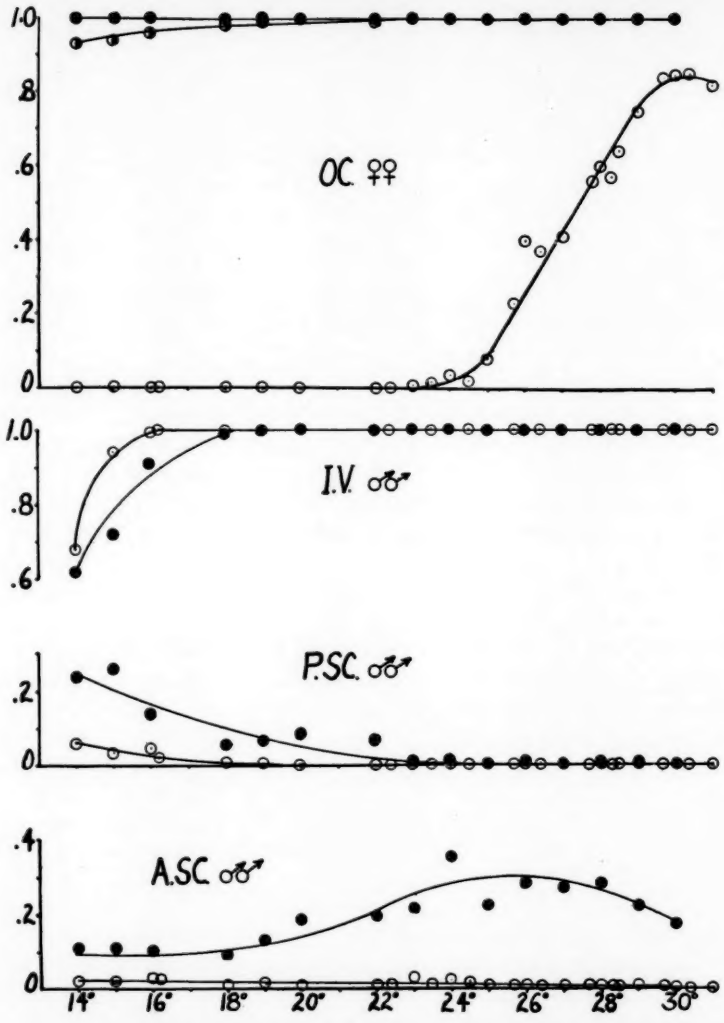

FIGURE 1.—Effect of temperature on mean bristle number. Ordinates, mean bristle numbers; abscissae, temperature in °C.

$\circ = sc\ 1 \qquad \bullet = \frac{sc\ 1}{sc\ 5} \qquad \bullet = sc\ 5$

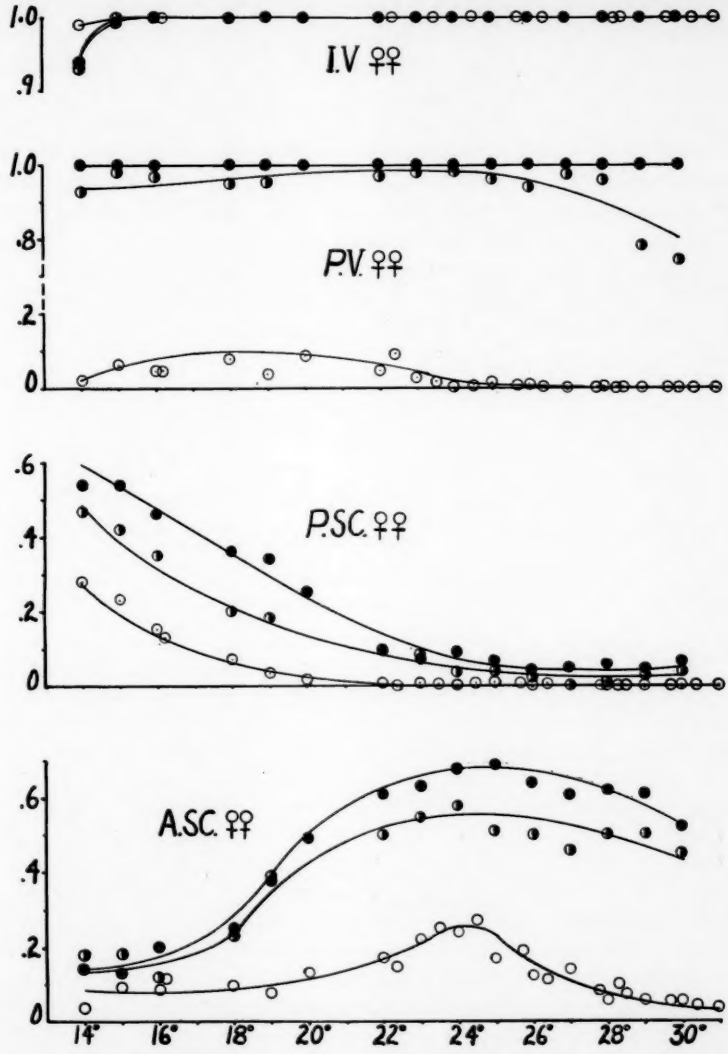

FIGURE 2.—Effect of temperature on mean bristle number. Ordinates, mean bristle number; abscissae, temperature in °C.

○ = sc 1 ◑ = $\frac{sc\ 1}{sc\ 5}$ ● = sc 5

on the head of the fowl. In populations homozygous for down defect there is a variation in the size of the bald spot, a certain percentage of the fowls not showing the character at all, although genetically down defect. A statistical analysis of such a population indicated that a definite percentage of "normal" fowl were to be expected, if one assumed a distribution in amount of "down defect producing substance" among the individuals of a homozygous down defect population. The "normal" fowl in this analysis had varying quantities of a smaller amount of "down defect producing substance" than the threshold amount needed to show the character.

In all these instances where the expression of a character depends upon a threshold amount of character producing substance, any amount less than the threshold will have no effect. An analogous situation may be seen

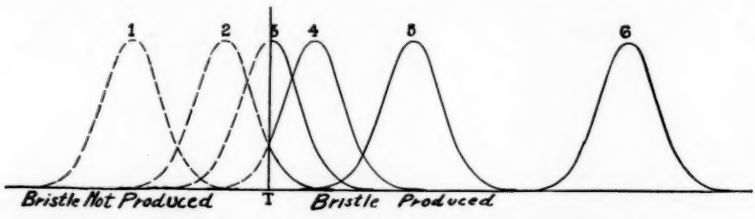

FIGURE 3.—Hypothetical distributions in concentrations of bristle producing substance for a particular bristle. The numbers 1–6 refer to various populations. T is the threshold concentration.

in muscle contraction where any subminimal stimulus evokes no response. If the character is variable such as wing or eye size, varying amounts of substance above the threshold may produce varying degrees of character expression. With respect to bristle production, however, the situation is more complex. A particular bristle is either present or absent, like the all-or-nothing principle in the contraction of a single muscle fiber. Any amount of substance above the threshold amount will produce only one bristle.

The effect of the threshold with respect to a bristle is illustrated in figure 3. Populations such as 2–4 will have mean bristle numbers between 0 and 1 (curve 2 near 0, curve 4 near 1). Populations which do not cross the threshold value will have mean bristle numbers of 0 if on the left or of 1 if on the right of threshold, although these populations differ from one another, for example 5 and 6.

In *sc* 5 (fig. 2) the oc bristle is never affected (is like the wild type) at all temperatures (like curves 5, 6 in fig. 3). In *sc* 1 the oc bristle is absent in all flies raised under 22° (like curve 1 in fig. 3). The M. oc increases rather sharply with increasing temperature (like curves 2–4). In *sc* 1/*sc* 5

the M. oc equals 1 above 20°, and is less than 1 below 20°. These results indicate that although the M. oc in *sc 5* is 1 at all temperatures, the concentration of bristle producing substance is not the same at all temperatures and similarly for *sc 1* below 22°. The p.v. bristle (fig. 2) behaves in a somewhat similar manner. The temperature effect on *sc 1/sc 5* indicating that either *sc 1/sc 1* and *sc 5/sc 5* or both have a decreasing amount of bristle producing substance with increasing temperature above 25°. In *sc 1 sc 1*, since the amount is already below the threshold (curve 1 and 2, figure 3), no further effect on the bristle number can be seen. In *sc 5/sc 5* the inference is that the amount of bristle producing substance although decreasing above 25° does not cross the threshold.

According to this interpretation, in order to use mean bristle number as an exact quantitative character in a study of the chemical kinetics involved in bristle production, a statistical or experimental analysis of one bristle and no bristle must be made. PLUNKETT (1926) found that because of the arbitrary assumptions needed in a statistical analysis, a satisfactory result could not be obtained. It is hoped that experimental studies with combinations of other scute alleles will shed more light on the problem.

A study of the temperature effective periods of these stocks is being made. Preliminary results indicate that both the temperature-effective periods and the time of development differ in these three stocks.

SUMMARY

1. The mean bristle numbers in isogenic stocks of *sc 1*, *sc 5*, and *sc 1/sc 5* were determined at temperatures ranging from 14° to 30°.

2. *Sc 1* is a more extreme allele than *sc 5* for all bristles affected except the i.v. bristle.

3. The effect of temperature on the mean bristle number is in the same direction in the three stocks.

4. The mean bristle number of *sc 1/sc 5* is intermediate between *sc 1* and *sc 5* at all temperatures.

5. The problem of thresholds as applied to the presence and absence of a particular bristle is discussed.

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STUDIES ON THE BAR SERIES OF DROSOPHILA
III. THE FACET RELATIONS IN DORSAL AND VENTRAL LOBES OF THE EYE

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INTRODUCTION

THE relative growth of parts of many organisms has been shown to conform to the simple power function

$$y = bx^k$$

where y and x represent the parts measured, and b and k are constants. When y and x are plotted on a logarithmic scale a straight line is obtained. The many applications of this equation to growth data have been fully reviewed by HUXLEY (1932). More recently, HERSH (1934) and ROBB (1935a, 1935b) have extended the application of the relative growth function from the purely ontogenetic sphere to the phylogenetic, through investigations on the evolutionary relative growth of parts in the Titanotheres and the horse, respectively.

The first attempt to apply the relative growth function to data on the relative sizes of parts in Drosophila was made by HERSH (1928). The well known lobing of the compound eye in Bar-eyed flies makes possible a quantitative determination of the relative sizes of the two lobes expressed in terms of facet number. In a series of investigations on Bar raised at different temperatures (HERSH 1928) and on different Bar stocks raised at the same temperature (HERSH 1931), HERSH concludes that the relative growth function adequately expresses the observed relations between facet number in the dorsal and ventral lobes of the eye.

In my own investigations on facet number in Bar (MARGOLIS 1935), separate records of facet number in the two lobes of the eye were kept in so far as possible. These data, together with others gathered subsequently onan unrelated Bar stock obtained from DR. A. L. BARON, serve as the material for the present analysis of the facet relations in dorsal and ventral lobes of the eye. My own results differ from those of HERSH in several respects, particularly with reference to the applicability of the relative growth function. At the same time, the data suggest a possible basis for the observed differences and are accordingly presented.

EXPERIMENTAL

Stocks

The data on facet number are taken from two unrelated but genetically homogeneous Bar stocks. The stock designated as A is the one used in

my earlier experiments on the effect of the gene vestigial on facet number in Bar. The details concerning inbreeding, genetic homogeneity, culture methods, facet number for the whole eye, etc., have been presented elsewhere (MARGOLIS 1935). The stock designated as B is the one obtained from Dr. A. L. BARON. This stock had previously been rendered isogenic with a wild Oregon stock used by Dr. POWSNER in his experiments on the duration of various developmental periods at different temperatures (POWSNER 1935). The general method of rendering the two stocks isogenic through the use of chromosomes with dominant markers and crossover suppressors has already been outlined (BARON 1935). Before using stock B in my own experiments, the stock was inbred with a vestigial stock by pair brother-sister matings for fifteen generations. This vestigial stock had previously been backcrossed to POWSNER's wild stock for 34 generations. This latter procedure was carried out in order to continue my earlier experiments on Bar and vestigial. The method insures a high degree of genetic uniformity in both the Bar and vestigial stocks and, at the same time, a close relationship to the wild stock of POWSNER. Since this latter stock has shown such excellent viability, fertility, and genetic homogeneity, a number of workers in our laboratory are using it as a standard wild-type with which the developmental effects of various mutant characters may be compared. The actual degree of genetic uniformity obtained in stock B by the above procedure, as measured by the parent-offspring correlation, will be discussed later.

Methods

The experimental methods employed in securing the data for stock A have been described elsewhere (MARGOLIS 1935). A series of seven experiments were conducted using several temperatures in each experiment, and extending over a period of a year and a half.

The data on facet counts in stock B were taken from a series of experiments dealing with a preliminary localization of the temperature-effective period for facet determination in this stock, and from other experiments dealing with the effect of reduced atmospheric pressures on facet number. None of the data on stock B have been published previously and are presented here strictly from the point of view of determining the relation between dorsal and ventral lobes of the eye in respect to facet number. The experiments on the effect of reduced atmospheric pressures proved negative and will be considered elsewhere as one aspect of the effects of various environmental agencies on facet number in Bar. The facts relating to culture methods, temperature control, and general uniformity of environmental factors are the same for stock B as for stock A.

Facet counts

In making the facet counts on both stocks, separate records of facet number in the two lobes were kept where possible. In the case of Bar there is always some indication of lobing of the eye; but in the larger eyes, especially at low temperatures, there is a tendency for the two lobes to merge, making accurate discrimination between lobes difficult. I found it extremely difficult to distinguish accurately between lobes at temperatures below 22°C, so that the data below this temperature are far too meager for analysis and are not given. At all temperatures, personal error in distinguishing between lobes was minimized by recording the lobes separately only in cases where clear definition was possible. A comparison of the total eye size of flies in which dorsal and ventral lobes were clearly distinguishable with the total eye size of the population as a whole, at each temperature, indicates that the former is truly a random sample of the latter. This fact is easily verified by comparing table 1 of this paper with table 3 in the paper previously cited (MARGOLIS 1935).

Data on facet number in dorsal and ventral lobes

Throughout this paper the mean facet number in the dorsal lobe at any temperature will be designated as D, and correspondingly the ventral lobe facet number as V. In table 1 are given the values of D and V, respectively, for different temperatures, together with their standard deviations. The column headed D/V gives the ratio of D to V and the column headed r, the correlation between lobes.

A number of interesting facts are apparent in the data. In those cases where experiments at a given temperature are repeated one or more times the values of D and V, in general, are similar within the limits of sampling error. The ratio, D/V, differs for the two sexes scattering narrowly around a value of 1.15 for the males and 1.0 for the females. For either sex the ratio is relatively constant over the temperature range. The values of the correlation coefficients (r) present the most interesting feature of the table. There is, in general, a fairly systematic decrease in the value of r with increase in temperature. At the same time, however, where experiments at a given temperature are duplicated there are in some cases significant differences in the values of r for any one temperature. This is especially noticeable in the case of the four experiments at 28°. This temperature was used as a control temperature throughout the series of experiments, and although the mean facet numbers display an insignificant scattering in the different experiments there are significant differences in r. This indicates an inadequate control of some factor or factors which influence variation in facet number in the two lobes, but which do not perceptibly shift the

TABLE 1

Statistics on facet number in dorsal and ventral lobes of stock A.

EXP.	SEX	T	D	σ_D	V	σ_V	D/V	r	n
IV	♂	22°	57.9±1.7	7.90	50.0±1.5	6.85	1.16	0.600±0.136	22
	♀	22°	51.2±1.3	6.60	51.6±1.3	6.60	0.99	0.279±0.181	26
V	♂	22°	58.6±2.3	9.20	52.0±2.0	8.10	1.13	0.902±0.047	16
	♀	22°	52.9±1.3	5.05	53.6±1.2	4.90	0.97	0.881±0.056	16
VII	♂	22°	58.2±2.1	8.85	48.0±2.1	8.85	1.21	0.690±0.124	18
	♀	22°	50.3±1.5	7.20	52.6±1.6	7.89	0.96	0.701±0.104	24
VII	♂	24°	45.4±1.3	7.26	37.9±1.1	6.39	1.20	0.515±0.129	32
	♀	24°	40.1±0.8	5.76	40.4±0.9	6.45	0.99	0.612±0.089	50
I	♂	25°	40.7±1.1	4.88	34.4±1.0	4.62	1.18	0.677±0.120	20
	♀	25°	38.6±1.0	5.34	35.7±0.8	4.29	1.08	0.555±0.131	28
VII	♂	25.8°	36.9±0.8	5.40	31.6±0.8	5.25	1.17	0.556±0.105	43
	♀	25.8°	35.5±1.1	5.31	34.7±1.2	5.49	1.02	0.680±0.114	22
II	♂	27°	31.3±0.8	4.40	27.2±0.6	3.52	1.15	0.448±0.145	30
	♀	27°	28.7±0.7	3.90	29.9±0.8	4.82	0.96	0.628±0.106	33
III	♂	27°	31.3±0.4	4.00	27.9±0.4	4.20	1.12	0.427±0.077	112
	♀	27°	28.6±0.4	3.92	29.1±0.5	4.26	0.98	0.398±0.090	88
II	♂	28°	27.3±0.6	4.18	24.3±0.5	3.54	1.12	0.634±0.081	53
	♀	28°	22.4±0.7	4.10	23.3±0.6	3.74	0.96	0.147±0.157	39
III	♂	28°	26.3±0.7	4.24	22.8±0.5	2.96	1.15	0.488±0.121	40
	♀	28°	26.4±0.7	4.08	22.3±0.6	3.40	1.18	0.404±0.146	33
IV	♂	28°	25.7±0.6	3.60	23.6±0.6	3.18	1.09	0.382±0.149	33
	♀	28°	25.4±0.8	4.48	25.0±0.7	3.68	1.02	0.224±0.173	30
VI	♂	28°	27.1±0.7	4.14	25.3±0.4	2.52	1.07	0.161±0.170	33
	♀	28°	23.7±0.8	3.78	25.8±0.2	3.66	0.92	0.164±0.203	23
I	♀	29°	22.6±0.8	3.24	19.8±0.6	2.44	1.14	0.063±0.249	16
III	♂	29°	22.0±0.5	3.40	20.2±0.4	2.46	1.09	−0.092±0.143	48
	♀	29°	20.9±0.5	3.06	19.0±0.4	2.38	1.10	−0.076±0.166	36
II	♂	30°	22.0±0.7	2.58	20.1±0.5	1.85	1.09	0.522±0.195	14
	♀	30°	19.8±0.6	2.24	18.7±0.4	1.68	1.06	0.017±0.250	16
III	♂	30.4°	17.3±0.5	2.35	14.8±0.6	2.87	1.17	0.225±0.194	24
	♀	30.4°	17.0±0.3	1.93	15.9±0.4	2.34	1.07	0.356±0.152	33
I	♀	31°	17.6±0.3	1.03	15.8±0.4	1.73	1.11	−0.231±0.231	17

mean value for the population. As will be seen later, this feature in the data appears also in stock B. There is, as one should expect, a close approximation in the values of r for the two sexes. The few exceptions to this fact will be considered in connection with the data in table 2.

The biometric constants for facet number on stock B are contained in

TABLE 2

Statistics on facet number in dorsal and ventral lobes of stock B.

EXP.	SEX	T	D	σ_D	V	σ_V	D/V	r	n
IIb	♂	22°	69.1±1.1	6.49	42.2±0.8	4.71	1.64	0.535±0.121	35
	♀	22°	48.3±0.9	5.85	42.9±0.8	4.91	1.13	0.252±0.146	41
IIc	♂	22°	79.7±1.3	6.45	49.6±0.9	4.50	1.61	−0.237±0.193	24
	♀	22°	49.0±0.9	4.08	44.1±1.3	5.81	1.11	0.332±0.199	20
IId	♂	22°	77.6±1.6	6.30	50.2±1.6	6.05	1.55	0.188±0.250	15
	♀	22°	47.8±0.8	4.56	43.2±1.2	6.77	1.11	0.221±0.165	33
IIa	♂	28°	43.4±0.6	5.72	26.2±0.3	3.31	1.66	0.192±0.092	110
	♀	28°	27.7±0.3	3.36	22.8±0.3	2.90	1.22	0.455±0.072	122
IIIa	♂	28°	44.4±0.8	6.38	27.8±0.5	3.80	1.60	0.391±0.106	64
	♀	28°	28.5±0.5	3.01	23.8±0.5	3.01	1.20	−0.190±0.145	44
IIIb	♂	28°	41.4±0.6	4.50	26.1±0.3	2.40	1.59	0.200±0.133	52
	♀	28°	29.6±0.6	3.79	23.2±0.5	3.08	1.28	0.250±0.154	37
IIIc	♂	28°	42.4±0.8	5.74	26.4±0.4	2.67	1.61	0.190±0.130	55
	♀	28°	29.9±0.5	3.08	23.0±0.5	3.17	1.30	0.251±0.159	35
IIId	♂	28°	41.2±0.9	5.87	25.3±0.4	2.70	1.63	0.156±0.143	46
	♀	28°	30.3±0.6	3.12	22.4±0.5	2.74	1.35	0.252±0.184	26
IIIe	♂	28°	45.2±1.0	6.92	26.5±0.5	3.48	1.71	0.350±0.125	49
	♀	28°	30.4±0.7	4.02	23.3±0.5	2.87	1.30	−0.048±0.166	36
IIIf	♂	28°	43.6±1.0	5.54	25.9±0.6	2.98	1.68	0.082±0.187	28
	♀	28°	30.6±0.8	3.88	23.0±0.5	2.63	1.33	0.141±0.192	26
IIIg	♂	28°	43.4±0.9	6.10	26.7±0.5	3.20	1.63	0.512±0.104	50
	♀	28°	32.8±0.7	3.72	25.0±0.8	4.52	1.31	0.276±0.168	30
IIIh	♂	28°	41.0±0.8	5.14	24.7±0.4	2.58	1.66	0.114±0.147	45
	♀	28°	30.8±0.6	3.70	22.5±0.5	3.18	1.37	−0.189±0.163	35
IIIi	♂	28°	46.0±0.9	4.26	25.6±0.7	3.14	1.80	0.107±0.206	23
	♀	28°	31.6±0.6	2.50	22.8±0.5	2.20	1.39	0.115±0.233	18
Ia	♂	28°	42.7±0.7	5.71	27.6±0.5	3.54	1.55	0.314±0.108	58
	♀	28°	29.0±0.6	3.00	24.0±0.6	2.90	1.21	0.188±0.182	28

table 2, and are arranged in the same manner as table 1. Only two temperatures are represented in the various experiments. Although data at other temperatures are desirable, these data in themselves clearly bring out certain features of the facet relations in dorsal and ventral lobes. In so far as duplication of means is concerned the experiments are entirely satisfactory with the exception of experiment IIb at 22°. The scattering in values of D and V at 28° does not appear to be significant statistically although there is a slight trend when the male and female values for different experiments are compared. This indicates some slight variation in experimental conditions from one experiment to another, too small to be detected in the sampling errors of the means in populations of this size.

The values of D/V differ for the sexes, although they appear to be relatively constant for the two temperatures in the males. There is some indication that D/V is lower at 22° than at 28° for the females. The correlation between lobes is, in general, small and in most individual experiments not statistically significant. Viewing the data as a whole there is no doubt that a small but significant positive correlation between lobes exists. The basis for this correlation may rest upon some residual genetic variability, upon some inadequately controlled environmental variable, or finally upon some sort of developmental interdependence between the two lobes. If development of facets is independent in the two lobes, then in a genetically homogeneous stock raised under uniform environmental conditions the correlation between lobes should be 0. In this event, the presence or absence of a significant correlation may be used as a test for the uniformity of all factors which determine facet number in any experiment.

An experiment was designed to test the degree to which genetic heterogeneity in stock B might account for the observed correlation between lobes. A parent-offspring correlation for facet number was carried out at 28°. In both the parents and the offspring separate records of dorsal and ventral lobes in left and right eyes were kept. Table 3 contains the data on the parent-offspring, left-right, and dorso-ventral correlations in stock B. The slight discrepancy in numbers of individuals on which each correlation coefficient is based is due to the previously mentioned fact that clear discrimination of lobes is not always possible, coupled with the fact that in some cases one or the other eye cannot be counted due to mechanical injuries of one sort or another.

The values of r for parent-offspring and for dorso-ventral lobes are very similar in both males and females. The small differences which appear are probably sampling errors. The left-right correlations, on the other hand, are much larger and differ significantly from the other correlation coefficients. This latter fact confirms my view that in Bar some form of developmental dependence exists between the two eyes (MARGOLIS 1935).

Since the parent-offspring correlation serves as a quantitative measure of the degree of genetic heterogeneity in a population, one may conclude that in this experiment genetic heterogeneity, in itself, is sufficient to account for the observed correlation between dorsal and ventral lobes. Environmental variations, if present, will increase the correlation. The same is true for any sort of developmental interdependence, as demonstrated by the left-right correlations.

The fact that residual genetic heterogeneity adequately accounts for the dorso-ventral correlation is further supported by the values of r at 28° in table 2. The great majority of these values in both sexes closely approximate the values of the parent-offspring correlations in table 3. The few exceptions which show high positive correlation may be due to some inadequately controlled environmental variable which escaped attention during the experiments.

TABLE 3

Parent-offspring, left-right, and dorso-ventral correlations in stock B taken from the same set of data.

MALES

r(parent-offspring)	$=0.188\pm0.060$; n = 256
r(left-right)	$=0.483\pm0.050$; n = 235
r(dorso-ventral)	$=0.273\pm0.061$; n = 228

FEMALES

r(parent-offspring)	$=0.246\pm0.069$; n = 182
r(left-right)	$=0.560\pm0.056$; n = 150
r(dorso-ventral)	$=0.196\pm0.074$; n = 170

Attention has already been directed to those cases in table 1 where a significant difference in r between males and females is observed. A few similar cases are present in table 2. It is perhaps noteworthy that, with a single exception (table 2, exp. IIa), wherever a large difference in r exists, the males show the higher value. Formally, at least, this fact can be explained by assuming the presence of sex-linked recessive modifiers of facet number which are not uniformly distributed in the population. The presence of sex-linked modifiers of Bar is known from the experiments of HERSH (1929). Such modifiers would affect the facet number in the males, but might be masked in the females through the effects of more uniformly distributed dominant alleles. An alternative explanation in terms of differential effects of environmental variables on the two sexes is tenable but appears less probable.

The systematic trend in r for stock A (table 1) is interesting in the light of the explanation offered here for the correlation observed between lobes. One should expect to find the values of r scattering about some central value representative of the parent-offspring correlation, if the interpreta-

tion given for stock B applies equally to stock A. The parent-offspring correlation for stock A at 28° was found to be 0.17 ± 0.067 for the males, and 0.25 ± 0.065 for the females. At 28°, with the exception of males in experiment II, the values of r do not depart widely from the values for the parent-offspring correlation. It is obvious that at all temperatures below 28° the values of r are much higher than one is led to expect from the parent-offspring correlation. This raises an important question as to whether a routine determination of a parent-offspring correlation with reference to any quantitative character under a single set of conditions, for example a single temperature, is sufficient for establishing the degree of uniformity of genetic factors affecting that character. From our knowledge of the effects of environmental agencies on development in general, it appears probable that parent-offspring correlations will differ under different sets of conditions. An experimental investigation of this point is highly desirable and will be undertaken. The trend in r with temperature in table 1 may be due to the presence of eye modifiers not uniformly distributed in the population. The effects of these modifiers may be progressively obscured as temperature increases, through the more efficient operation of processes initiated by other genes which are more uniformly distributed. This explanation is, of course, purely formal but can be tested by investigating the parent-offspring and dorso-ventral correlations over a range of temperatures.

THEORETICAL

HERSH (1928) has investigated the inter-lobe correlation for a number of combinations of *B*, *BB*, and wild type over a range of temperatures. For comparison with my own results the data on *B* are most relevant. The correlation between lobes is moderately high at most temperatures and shows no systematic trend with temperature. The stock used in those experiments is the low selected forked Bar stock, obtained from ZELENY in 1921, and used by HERSH in other investigations (HERSH 1924 and 1927). No measure of genetic uniformity in the stock is available since no parent-offspring correlation is given. In view of the rigorous selection for low facet number in this stock, a high degree of uniformity may be assumed. However, a comparison of the facet temperature relation in 1924 with that in 1927 (compare table 3, HERSH 1927 with table 1, HERSH 1924) indicates that some germinal modification had taken place during the years following selection and inbreeding. This is indicated by the relatively higher count at all comparable temperatures in 1927. I have found in my own stocks a marked tendency toward accumulation of high modifiers following close inbreeding for many generations. This progressively accumulated genetic diversification is, perhaps, sufficient to account for the inter-lobe correlation.

Moreover, the use of a 3 to 4 day egg-laying period introduces an environmental variable which operates differentially on the flies in any one culture. Those flies developing from the early laid eggs are exposed to optimum conditions, whereas the flies from later eggs show effects of progressive depletion of food supply as evidenced by a decrease in facet number and general body size (LUCE 1931, MARGOLIS 1935). Data to be published elsewhere demonstrate clearly that poor culture conditions increase the inter-lobe correlations. PEARL (1906) observed a small but similar increase in size correlations in Chilomonas due to poor culture conditions. It is, of course, impossible to establish the degree to which the various factors mentioned contribute to the inter-lobe correlations observed, unless the genetic and environmental factors are controlled separately.

An interesting point of difference in HERSH'S 1928 data and my own, is in the relation of the facet ratio between lobes (D/V) to temperature. As illustrated in table 1 for stock A, D/V is constant for all temperatures, for both sexes. HERSH, on the other hand, finds that when log D is plotted against log V for each temperature a straight line is obtained. This, of course, means that $\frac{\log D}{\log V}$ is constant over the range of temperatures. From this fact HERSH concludes that the rate of formation of facets in the dorsal and ventral lobes is logarithmic in character. This analysis, however, tells us nothing concerning the rate of formation of facets in the individual under any specified set of conditions, but gives us the relation of facet number to temperature for dorsal and ventral lobes, respectively. This can be illustrated more clearly in the following manner. HERSH (1930) has shown that the facet-temperature relation for various combinations in the Bar series is exponential in character, conforming to the expression

$$y = ae^{rt}$$

where y is the number of facets, t the temperature, a and r constants, and e the base of the system of natural logarithms. This expression gives an excellent fit to the data. If now, both the dorsal and ventral lobes are exponential functions of temperature such that

$$D = ae^{rt} \quad (1)$$

$$V = a^1e^{r^1t} \quad (2)$$

then solving (1) and (2) in terms of t and eliminating t and e from the equations, D is related to V as a power function of the form

$$y = bx^k \quad (3)$$

where $y = D$, $x = V$, and b and k can be evaluated in terms of the constants

a, a^1, r, and r^1 in equations (1) and (2). This is, in fact, implicit in plotting log D against log V for different temperatures as carried out by HERSH. In plotting the data as indicated above it is temperature and not time which is implicit, so that no growth relation may be assumed.

Since, in my own data (table 1), it is $\frac{D}{V}$ rather than $\frac{\log D}{\log V}$ which is constant, some explanation for the difference is desirable. Considering again equations (1) and (2), in the special case where the constants r and r^1 are equal, the constant k in equation (3) which is equal to r/r^1 becomes equal to 1 so that the relation between D and V over the temperature range is rectilinear. It appears then that D and V in both HERSH's and my own data are related to temperature in the same manner but that HERSH's results conform to the more general relation of which my own represent a special case, for example when r and r^1 are equal.

In a further investigation of the facet relations in the dorsal and ventral lobes, HERSH (1931) has fitted the relative growth function to data on a series of Bar stocks differing from each other in respect to various mutant markers in different regions of the X chromosome. The experiment was conducted at 25° C. In applying the relative growth function to these data, HERSH points out that there is the assumption that increasing facet numbers in the two lobes represent progressive growth levels. Fifteen stocks were tested, some giving an excellent fit to the calculated curves, others a questionable fit, and still others rather wide departures. This statement is based merely on visual inspection of the curves rather than on any tests of goodness of fit.

The manner in which the facet numbers in dorsal and ventral lobes were calculated requires some consideration. In making these calculations for any one series, the facet data were seriated on the basis of total facet number instead of treating facet number in dorsal and ventral lobes as independent variates. The latter procedure is the one used in determining regression, and involves no assumptions concerning the relations of the variates. In his 1928 paper HERSH states that regression for those data was sensibly linear. If regression is likewise linear for the 1931 data, it is clear that the relative growth function cannot satisfactorily be fitted to data seriated with reference to either dorsal or ventral lobes treated as independent variates. It is of some interest, then, to determine the consequences of seriation of data on the basis of total eye size. Expressed in general terms the problem may be stated as follows: given two variables, D and V, such that $D+V=T$, assuming normal distributions for D and V, respectively, and o correlation, what will be the relation between D and V calculated from data seriated with reference to T?

This problem was attacked by building up a series of theoretically con-

structed correlation tables for D and V using the normal distribution, and assuming 0 correlation. Different relative magnitudes of the standard deviations and means were used for the two distributions. Analysis of these tables showed that for data seriated with reference to T there is a well defined relation between D and V despite the absence of any correlation. When the standard deviations for D and V differ, the relation is sigmoid; when the standard deviations are equal, the relation is rectilinear. The values of the means, however, do not affect the form of the relation.

It was of further interest to test the application of these conclusions to a set of data which approximated the conditions specified in the analysis, namely, normality of distribution of the variates and 0 correlation. For this purpose the data on facet number for the females of stock B, from experiments IIIa, IIIc, IIId, IIIe, IIIf, IIIh, and IIIi, were used (table 2). The data from the different experiments were then grouped together and biometric constants for dorsal and ventral lobes calculated. The values for the constants are as follows:

$$D = 30.10 \pm 0.237;\ \sigma_D = 3.484$$
$$V = 23.08 \pm 0.197;\ \sigma_V = 2.900$$
$$r = -0.0092 \pm 0.0680$$
$$N = 215$$

The data were then seriated with reference to total eye size and values of D and V calculated for each class. These values of D and V are plotted in figure 1, and are represented by open circles. The solid circles in figure 1 represent the values of D and V calculated from the same data, but seriated with reference to D. The latter represents graphically the regression of V on D calculated in the usual way. The curve in figure 1 represents a theoretially calculated curve using the means and standard deviations of dorsal and ventral lobes respectively and assuming normality of distribution of the variates as well as o correlation. The horizontal line represents the theoretical regression of V on D. The sigmoid character of the curve calculated from data seriated with respect to total facet number is clearly apparent. Were it not obvious that this is a consequence of the mathematical treatment of the data, one might be led to assume some functional relation between facet numbers in the two lobes. The absence of any such relation is, however, clearly indicated by the analysis of the data seriated with reference to either dorsal or ventral lobes, and by the absence of correlation between lobes. Considering the nature of the assumptions involved in the calculation of the theoretical curve, a remarkably good fit is obtained. The first point, for example, is based on only one individual. The whole question of the form of distribution of facet number in Bar will

be treated elsewhere, although the present data indicate an excellent approximation to normality.

One must conclude, at least for the data at 28°, that facet number in Bar is independently determined in the two lobes. The factors which lead

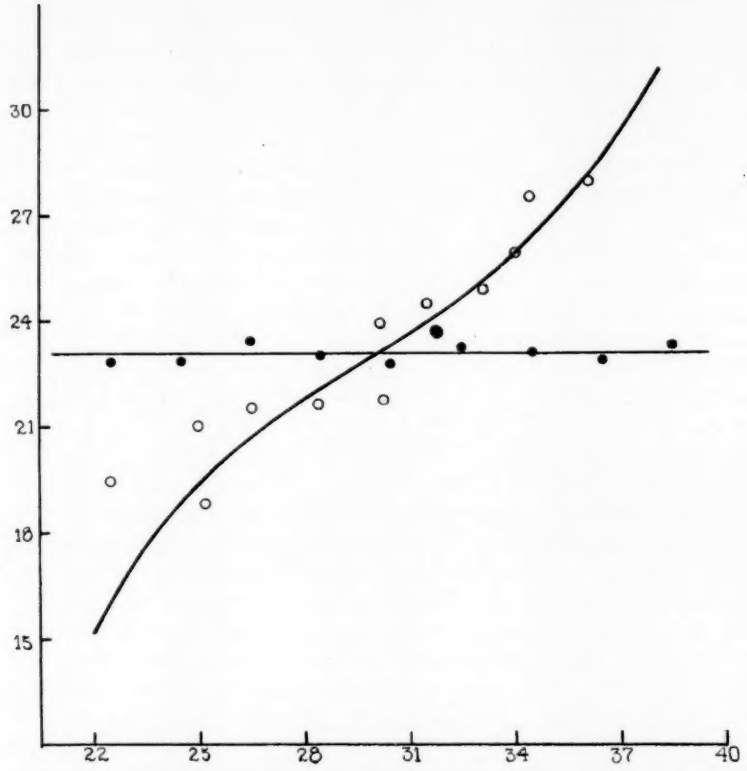

FIGURE 1. Ordinates—ventral facet number; abscissae—dorsal facet number. Solid circles represent data seriated with reference to facet number in dorsal lobe. Open circles represent data seriated with reference to total facet number. See text for calculation of curves.

to the impression of some degree of interdependence as evidenced by correlation between lobes have already been discussed at some length but may be summarized briefly.

1. Genetic heterogeneity in the population leads to a correlation between lobes since, although the lobes may vary independently of each other, the two lobes are covariant with respect to the genotype.

2. Environmental fluctuation operates in a similar manner. Facet num-

ber in either lobe varies with such factors as temperature, culture conditions, etc., so that variability in any one of these factors leads to concomitant variation in both lobes, in this way bringing about a correlation between lobes.

If this interpretation of the facet relations in the two lobes is correct, then we have a very simple method for testing the uniformity of both genetic and environmental factors in any experiment. Zero correlation should indicate uniformity of all factors affecting facet number. In this respect, the determination of inter-lobe correlations serves the same purpose as the left-right correlations for bristle number as used by PLUNKETT (1926) and subsequently by CHILD (1935). In the case of facet number, the left-right correlations are misleading since my data indicate some degree of developmental interdependence.

The question arises concerning the factors determining the lobing of the eye. DIETRICH (1907) has called attention to the fact that lobing is by no means uncommon among the Diptera. Moreover, this lobing is some times accompanied by differences in size of facets as well as histological pattern of the ommatidia in the two lobes. These facts are of interest since in Drosophila, superficially at least, there is no indication of lobing in the wild type eye, so that the Bar gene would seem to have introduced the lobing effect as a new feature. Recent unpublished observations of C. W. ROBERTSON of this laboratory, however, indicate that the ommatidia of the wild type eye are divisible histologically into two groups based upon the arrangement of the retinulae cells within each group. It appears then that the lobing in Bar is foreshadowed in the wild type, although the latter gives no superficial indication of this fact.

The apparent independence of facet number in the two lobes may be explained by at least two simple hypotheses. (1) The facet number in each lobe may be determined in a separate center. (2) Facet number may be determined as a whole, but an independent set of factors may operate to determine the lobing. If the factors determining the lobing operate independently of those determining facet number, then no correlation between lobes is to be expected. There appears at present to be no way of distinguishing definitely between these possibilities.

The observations and analysis of data on dorsal and ventral lobes of the eye in Bar-eyed Drosophila presented here show definitely that a number of factors may operate to produce an apparent relation between facet numbers in the two lobes where, in fact, no demonstrable functional relation exists. It is impossible to specify the manner in which each of these factors affects the end result, without experiments directed specifically toward that end. It is, however, clear that these factors should be evaluated in any analysis of the problem.

SUMMARY

Data on facet number in the dorsal and ventral lobes of the compound eye of Bar-eyed Drosophila are presented. Two unrelated but approximately genetically homogeneous stocks were used in the experiments. In stock A, the value of the correlation between lobes increases with decrease in temperature. This is interpreted as due to some residual genetic heterogeneity. In stock B, a series of experiments at 28°C indicate that the dorsoventral correlation is of the same magnitude as the parent-offspring correlation for the stock. It is concluded that genetic heterogeneity is adequate to account for the correlations observed. Variability in environmental agencies such as temperature and culture conditions also contribute to an increase in correlation between lobes. If these conclusions apply equally to all Bar stocks then the degree of correlation between lobes serves as a measure of uniformity of all factors, both genetic and environmental, which affect facet number.

An analysis on the effects of different methods of seriation of the data is presented. It is demonstrated that seriation of the facet data on the basis of total eye size leads to the impression of a functional relation between dorsal and ventral lobes where, in fact, no demonstrable relation exists, as evidenced by complete absence of regression or correlation.

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STUDIES ON HYBRID STERILITY IV. TRANSPLANTED TESTES IN DROSOPHILA PSEUDOOBSCURA

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INTERSPECIFIC hybrids may be equal or superior to their parents in somatic vigor and, at the same time, show disturbances in the gonads of such a nature that no functional gametes are produced. This antithesis between the general vigor on one hand and the degeneration of the reproductive tissues on the other constitutes an interesting developmental problem. In previous papers of this series (DOBZHANSKY 1934, 1936) it has been pointed out that there are two types of hybrid sterility, the "chromosomal" type in which meiosis is abnormal because of the structural noncorrespondence of the two parental sets of chromosomes, and the "genic" type in which sterility is caused by interactions of complementary genes contributed by both parents. In the chromosomal type the phenomenon of sterility is an intracellular one. In the case of genic sterility, on the other hand, it is possible, *a priori*, that intercellular influences are involved. One might suppose, for example, that there are diffusible substances, not characteristic of the parental species, produced in the hybrid, and that such substances have specific effects on the reproductive system. The work of IRWIN and COLE (1936 a, b, c) on hybrids of doves and pigeons has in fact demonstrated, by means of immunogenetic reactions, the existence in the erythrocytes of such "hybrid" substances, that is, of specific substances present in the hybrid but absent in the parents. Of course, the hypothesis of the intercellular causation of sterility does not necessarily involve the assumption of specific hybrid substances. The fate of a gonad may be determined by the general metabolism (in the broad sense of the term) of the body containing it.

Transplantation of hybrid gonads to the parental forms, and *vice versa*, provides a direct method for testing the above hypothesis. By means of appropriate transplants, it is possible to determine whether hybrid sterility, in a particular case, is determined by the constitution of the gonad itself (autonomous development), or whether the genetic constitution of the surrounding tissues is a contributing factor (dependent development). Races A and B of *Drosophila pseudoobscura* provide favorable material for such studies. The F_1 males from interracial crosses are fully viable and vigorous, but show profound and characteristic disturbances in their spermatogenesis and are completely sterile. Except for the testes, the reproductive system appears to be quite normal.

MATERIAL AND METHODS

The technique of transplantation developed by EPHRUSSI and BEADLE (1935, 1936) for Drosophila has been used successfully by these workers for transplanting larval testes of *D. melanogaster* (unpublished). In case of *D. pseudoobscura* special thin-walled large-bore micro-pipettes were employed, but otherwise the technique was essentially the same as described for *D. melanogaster*. Larval testes must be handled with a good deal of care to avoid rupturing the delicate external membrane. Unless otherwise stated, the transplantations described below were made with larvae approaching the prepupal stage.

Four strains of *D. pseudoobscura* were used: wild type strains Zuni-5 (race A, strong), and Seattle-6 (race B, moderately weak), and mutant strains eosin magenta short (race A, rather strong), and scutellar dela (race B, intermediate). The two latter strains carry mutant genes affecting the eye color as well as that of the testicular envelope. The experiments were so arranged that it was possible to distinguish the implant from the testes of the host by color. Testis color in these experiments proved to develop autonomously, although non-autonomous development of this character has been observed in *D. simulans* (DOBZHANSKY 1931).

Eggs were collected over 24 hour periods at room temperature (about 22°C), then allowed to develop and hatch at 25°. Approximately 48 hours after collection, larvae were transferred to culture dishes and, from that time until transplantations were made, were grown at 19°. Larvae on which operations had been made were allowed to develop to maturity at 25°. In many cases, it was necessary to provide food after the operations; this was done by adding to the vial a small piece of standard food seeded with yeast.

The implanted testes, and in some cases those of the host, were fixed and stained in aceto-carmine, and smear preparations made. Spermatogenesis in normal and hybrid males has been described previously (DOBZHANSKY 1934). The difference in spermatogenesis between hybrid and normal males is characteristic enough, so that relatively slight modifications of either are readily detectable. In the hybrids the chromosomal pairing at meiosis is more or less suppressed, no second spermatocytes are formed, and the spermatids undergo a characteristically abnormal development. All of these abnormalities were looked for in preparations of the implanted testes and in the testes of the host, especial attention being paid to chromosome pairing at diakinesis and metaphase of the first meiotic division, since the process of pairing is known to be sensitive to external agents (BAKER 1936, BAUER, unpublished).

DEVELOPMENT OF IMPLANTED TESTES

Among 146 individuals in which testes were implanted in the larval stage, 22.8 per cent contained no implant when dissected, presumably because of loss or injury to the testis during the process of injection. Among those flies in which the implant had developed 61.1 per cent had the implant lying in the body cavity not connected with the reproductive organs of the host except by tracheal branches, 27.3 per cent had the implanted testis attached to one of the two vasa efferentia and one of the host's testes free in the body cavity, and in 11.5 per cent both the implant and one of the host's testes were attached to the same vas efferens (fig. 1).

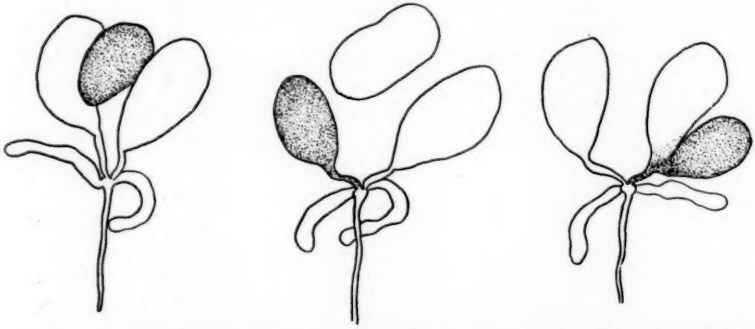

FIGURE 1.—The internal reproductive organs of eosin magenta males with A ♀ × B ♂ hybrid testes implanted in them. The implanted testes are in this case red (stippled in the figure), and those of the host pale yellow (white in the figure). Left—both host's testes attached to the vasa efferentia, the implanted testis free. Middle—the implant attached, one of the host's testes free. Right—implanted and host testes attached to the same vas efferens.

The connection established between the implanted testis and the vas efferens of the host may become functional, as shown by the fact that the sperm from the implant may be used in fertilization (see below). When two testes are attached to the same vas efferens it is not known whether the connection is functional for both of them. The data indicate that race A testes implanted in race B hosts become attached to the ducts of the host more frequently than race B testes in race A hosts; however the numbers of individuals are so small that this conclusion cannot be established with certainty. In the hybrids in which the testes of the host are small and the implanted testis of one of the pure races is much larger, no tendency is observed for the implant to be attached in preference to the host's testes.

In a control experiment testes of eosin magenta short (race A) were implanted in Zuni-5 (race A) larvae. Spermatogenesis in the implanted testes was found to be normal, showing that the process of transplantation as

such does not affect the normal development of the testis. During the entire study preparations of testes found lying free in the body cavity were kept separate from those of testes attached to the ducts; no difference was found in spermatogenesis in these two classes. Furthermore, in some cases testes were implanted into female hosts. A testis which develops in a female body is somewhat smaller in size than one which develops in a male. A similar observation was made in the case of certain types of gynandromorphs in *D. simulans* (DOBZHANSKY 1931). In case of attachment to a female duct no study of spermatogenesis was made, but no abnormalities in spermatogenesis were detected where the testis was not attached to an oviduct.

TRANSPLANTS BETWEEN RACES

Nine Zuni (race A) males with dela (B) testis implants, and seven eosin magenta (A) males with Seattle (B) implants were studied cytologically. Spermatogenesis was found to be quite normal in all stages. In 215 first spermatocytes, the normal number of bivalents, namely four, was observed. *D. pseudoobscura* has five pairs of chromosomes, but the fifth pair is so small that the bivalent formed by it is not usually seen at the meiotic stages. In one cell the X and Y chromsomes were unpaired, but such failure of pairing is occasionally observed in normal testes (DARLINGTON 1934). In the testes of the host spermatogenesis was observed to be normal. In 85 first spermatocytes, four bivalents were observed in each, and in one cell there were three bivalents and two univalents (X and Y chromosomes). One cyst of tetraploid spermatocytes was found. Six dela (B) males with Zuni (A) implanted testes also showed normal spermatogenesis in both the host and the implant. Four bivalents were seen in 99 first spermatocytes in the implanted testes and in 29 spermatocytes in the host.

In order to demonstrate that spermatogenesis is not only visibly normal but also leads to the production of functional sperm, eosin magenta males with Seattle implants were crossed to eosin magenta females. Nine out of ten such crosses produced offspring, which in six cases consisted of eosin magenta individuals only, and in three cases of eosin magenta as well as wild type individuals. Clearly, in the six former only the testes of the host, and in the three latter both the host's and the implanted testes functioned. Dela males with Zuni testes implanted were crossed to dela females; one out of four cultures produced only dela flies, and the remaining three both dela and wild type flies. The cases where no offspring were produced from the implanted testes are presumably those in which the implanted testis did not become attached to the vasa efferentia. The conclusion is justified that a race A testis developing in a race B body neither loses its ability to

function nor produces any disturbance in the testes of the host. The same is true for race B testes implanted in race A.

IMPLANTS FROM PURE RACES TO HYBRIDS

Four experiments of this type were made: (1) eosin magenta A testes were implanted into Zuni A ♀ ×Seattle B ♂ hybrids, (2) dela B into Zuni A ♀ ×Seattle B ♂, (3) eosin magenta A into Seattle B ♀ ×Zuni A ♂, and (4) dela B into Seattle B ♀ ×Zuni A ♂. In race B ♀ ×race A ♂ hybrid males, the testes are visibly smaller than in either pure race or in A ♀ ×B ♂ hybrids. Therefore, in the third and fourth experiments the implanted testes proved to be much larger than those of the hosts, while in the first and second experiments the implants were of the same or of slightly smaller size than those of the hosts. The implanted testes were studied cytologically and all stages of spermatogenesis proved to be normal. In the first experiment ten testes were examined and 54 first spermatocytes with four bivalents in each were seen; in the second experiment eight testes were examined and 68 spermatocytes with four and 1 with three bivalents (X and Y unpaired) found; in the third experiment five testes were examined and 76 cells with four bivalents found; in the fourth experiment eight testes were examined and 49 cells with four and 2 cells with three bivalents seen (X and Y chromosomes unpaired in one cell and an autosome unpaired in another).

About 12 males from each experiment were crossed to females homozygous for the recessive genes carried by the implanted testes. Since the hybrid males are normally sterile, the production of offspring from these matings would mean either that the implanted testes are able to function or that the presence of the implant induces fertility in the testes of the host. The presence of mutant genes as markers enables one to discriminate between these possibilities. Two males from each of the first, third and fourth experiments proved to be fertile, and the offspring showed that the sperm must have come from the implanted testes. We can conclude: (1) that the functioning of the testes of the pure races is not interfered with when they develop in the body of a hybrid, (2) that the testes of a hybrid host do not become fertile in the presence of a normally developed implant, and (3) that the ducts of the reproductive system in the hybrid males are normal and potentially functional.

IMPLANTS FROM HYBRIDS TO PURE RACES

Four experiments were made in which testes from hybrid males were implanted in males of pure races: testes of Seattle B ♀ ×Zuni A ♂ hybrids in (1) eosin magenta A males and (2) dela B males, and testes of Zuni A ♀ ×Seattle B ♂ hybrids in (3) eosin magenta and (4) dela males. In all

cases autonomous development of the implant was observed. The B ♀ ×A ♂ hybrid testes were small, and those of the A ♀ ×B ♂ hybrids were about equal in size to testes of the pure race hosts. In the first, second and fourth experiments seven implants were studied cytologically; in the third experiment three testes were studied. Spermatogenesis was found to be similar to that normally observed in hybrid testes; disturbance or complete lack of meiotic pairing, absence of second spermatocytes, degeneration of the spermatids, and other characteristic abnormalities. Four testes of the hosts were also examined, and found to contain normal stages of spermatogenesis. Five males from the first experiment were tested for fertility, and all proved to be fertile. It follows that the presence of a hybrid testis in the body of a normal male induces no change either in the implant or in the host.

TRANSPLANTATION AT EARLIER STAGES

In the experiments described above, both the donors and the hosts were nearly mature larvae (since the results were uniform, data regarding the precise ages of larvae in each individual experiment have been omitted). We have seen that under these conditions the development of the implanted as well as of the host testes is autonomous. It follows that in the mature larvae the fate of the testis is already determined, in the sense that its transplantation into the body of a foreign race does not produce any effect on spermatogenesis. The possibility remains however that if the transplantations were made at an earlier stage the results would be different. As an analogy, the findings of BAKER (1935) may be mentioned here. BAKER has shown that there is a sensitive period for the influence of high temperature on the testes of *D. pseudoobscura*, and that this sensitive period lies in the prepupal stage, that is, precisely the stage at which the majority of our transplantation experiments were made.

In the following two experiments the donor and the host larvae were much younger than in those experiments reported above, namely three days before pupation, which corresponds to the end of the second or the beginning of the third larval instars. After the operations were made, the larvae were given food sufficient to cover the period to pupation. The experiments were: (1) implants of Zuni A and (2) of Seattle B testes in dela B ♀ ×Zuni A ♂ hybrid larvae. Cytological examinations were made on five testes from the first experiment and on seven testes from the second. All stages of spermatogenesis were found to be normal: 72 spermatocytes in the first and 64 in the second experiment were found to contain four bivalents each; one testis from the first experiment contained a cyst of tetraploid cells, and in another testis a spermatocyte with only three bivalents (X and Y unpaired) was found.

About a dozen males from each of the two experiments were tested for fertility by crossing them to eosin magenta and to dela females respectively. Three males from the first, and none from the second experiment proved fertile. This low frequency of fertile males is due to the fact (ascertained by dissection) that in the large majority of males the implanted testis was not attached to the vasa efferentia.

DISCUSSION

Only few transplantation studies have been made which bear on the question of hybrid sterility in other insects. BYTINSKI-SALZ (1933) transplanted the ovaries of the pupae of the hybrid *Celerio euphorbiae* ♀ × *C. gallii* ♂ into pupae of the parental species. Normally the ovaries of the donor do not develop to maturity, since the hybrid does not survive the pupal stage; but the transplants were observed to grow and to form nearly mature eggs. It remains unclear whether in these experiments a transformation of a sterile gonad into a fertile one is involved, or (which seems vastly more probable) merely a survival of an organ which would normally die together with the organism containing it. EPHRUSSI and BEADLE (1935) transplanted the ovaries of *Drosophila simulans* to *D. melanogaster*, and found that the implant produces functional ova (the hybrid between these two species is completely sterile). Thus, the development of the ovary of one species in the body of another does not lead to a condition resembling hybridity. EPHRUSSI and MONOD (unpublished) transplanted the ovaries of *D. melanogaster* into *melanogaster* × *simulans* hybrid larvae. The resulting adult flies have three ovaries; two ovaries of the host are rudimentary (as they normally are in the hybrid), while in the implanted ovary apparently normal ova are present. The conclusion is that the ovary of *melanogaster* is not affected by development in the hybrid organism.

The data presented in the present paper are consistent throughout. The testes of one pure race develop normally in the body of the other race and in the hybrids, and the hybrid testes develop autonomously in the body of either race. Cytological investigation shows that there is no detectable influence of the host on the implant. Pure race testes produce functional sperm in the hybrid body, and the hybrid testes never develop fertility when implanted into the pure races. The development of the testes is autonomous, "herkunftsgemäss" and not "ortsgemäss." It follows that the condition of the gonad is determined by the genetic structure of the cells within the gonad and not by interactions between the gonad and influences emanating from other parts of the body. The only escape from this conclusion is to suppose that in our experiments the transplantations were made after the period during which the gonad may be sensitive to the influences of other parts of the body has passed. Such a supposition

has little chance to be correct, not only because some of the experiments were made in rather young larvae, but also because in *D. pseudoobscura* the proliferation of the primary spermatogonia continues during the whole of the pupal and large part of the adult life. At the stage at which the transplants were made the gonads contain mainly spermatogonia and a few spermatocytes. In other species of Drosophila, notably in *melanogaster* and *simulans*, the situation is different in this respect.

The phenomenon of sterility in the interracial hybrids in *D. pseudoobscura* must be intragonadal in nature. It would seem that only those external influences can modify the behavior of the sex cells which can act directly on the gonads. No "somatic induction" is likely to occur. This consideration is of importance for planning experiments which have to do with the physiology of sterility. It may be noted here that in cases where the sterility is of the chromosomal type (DOBZHANSKY 1934, 1936) the phenomenon is almost of necessity an intracellular one; but where genic sterility is concerned both intra- and intercellular causations are *a priori* equally possible. Hence, the results of this investigation of *D. pseudoobscura* cannot be generalized further. It should also be noted that transplantation of gonads between species may be expected on *a priori* grounds to produce in some cases a suppression of the implants. Such a suppression may not mean however that a condition resembling hybrid sterility is induced, unless it is demonstrated that cytological abnormalities resembling those found in sterile hybrids are apparent.

SUMMARY

Larval testes of race A were implanted into race B larvae, and *vice versa*. Testes of A×B hybrids were implanted into the pure races, and pure race testes were implanted into hybrids. Study of spermatogenesis has shown that the development of the implanted testes as well as of those of the host is autonomous in all cases. It follows that in *Drosophila pseudoobscura* the structure of the gonad is determined by its own genetic constitution and not by the genetic constitution of the surrounding soma.

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CORRIGENDA

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Frontispiece, photograph of John Belling was taken by Julian Scott.
Page 49, Table 4, last line, sixth column, for "7·33" read "73·3."
Page 150, line 6, second column, for "X20, X25" read "X20X25."
Page 152, line 20, for "early in the year" read "early in summer.'
Page 153, Literature cited, before "Fac. Sci." insert "J."
Page 160, line 33, after "cited" add "in part."
Page 170, table 4, column A, delete "p = 2/3."
Page 174, table 8, column A, for "chromatin" read "chromatid."
Page 176, lines 33 and 35, for "2^r X" read "X/2^r."
Page 181, line 23, for "non-modal" read "non-nodal."
Page 197, line 13, after "progressive" insert "and digressive."
Page 229, line 4, heading of table 1, for "male in female" read "female in male"; for "female in male" read "male in female."
Page 293, line 8, for "individual" read "cross"; for "individuals" read "crosses."
Page 286, Figure 1, for "natural size" read "$\frac{3}{4}$ natural size." Figures 6, 7, 8 for "5X" read "3.8X."
Page 382, line 26, for "auto" read "allo"; for "allo" read "auto."
Page 401, line 19, for "noted as being" read "noted being."
Page 402, in heading for "$\overline{X}^2$" read "X^2."
Page 499, at end of line 7, insert "(Table 2)."
Page 500, line 37, for "383 Pr bm_1" read "383 Bm_1 Pr."
Page 519, line 6, for "1927a" read "1927."
Page 519, line 14, for "1927b" read "1927."

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